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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :		(11) International Publication Number:
C12Q 1/68, C07K 14/72, 14/575, C12N 9/48	A2	WO 98/45477
		(43) International Publication Date: 15 October 1998 (15.10.98)

(21) International Application Number: PCT/IB98/00475

(22) International Filing Date: 1 April 1998 (01.04.98)

(30) Priority Data:
60/042,930 4 April 1997 (04.04.97) US

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CY, CZ, DE, DK, EE, ES, FI, GB, GE, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: METHODS FOR ASSESSING CARDIOVASCULAR STATUS AND COMPOSITIONS FOR USE THEREOF

(57) Abstract

The present invention provides methods for assessing cardiovascular status in an individual, which comprise determining the sequence at one or more polymorphic positions within the human genes encoding angiotensin converting enzyme (ACE), angiotensinogen (AGT), and/or type 1 angiotensin II receptor (AT1). The invention also provides isolated nucleic acids endoding ACE, AGT, and AT1 polymorphisms, nucleic acid probes that hybridize to polymorphic positions, kits for the prediction of cardiovascular status, and nucleic acid and peptide targets for use in identifying candidate cardiovascular drugs.

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METHODS FOR ASSESSING CARDIOVASCULAR STATUS AND COMPOSITIONS FOR USE THEREOF

This application claims priority under 35 U.S.C. § 119 from provisional U.S. application serial no. 60/042,930, filed April 3, 1997.

Field of the Invention

The present invention relates to genetic polymorphisms useful for assessing cardiovascular status in humans.

Background of the Invention

The renin-angiotensin-aldosterone system (RAAS) plays an important role in cardiovascular physiology in mammals. Specifically, RAAS regulates salt-water homeostasis and the maintenance of vascular tone. Stimulation or inhibition of this system raises or lowers blood pressure, respectively, and disturbances in this system may be involved in the etiology of, for example, hypertension, stroke, and myocardial infarction. The RAAS system may also have other functions such as, e.g., control of cell growth. The renin-angiotensin system includes at least renin, angiotensin converting enzyme (ACE), angiotensinogen (AGT), type 1 angiotensin II receptor (AT1), and type 2 angiotensin II receptor (AT2).

AGT is the specific substrate of renin, an aspartyl protease. The human AGT gene contains five exons and four introns which span 13Kb (Gaillard et al., *DNA* 8:87-99,

1989; Fukamizu et al., *J.Biol.Chem.* 265:7576-7582, 1990). The first exon (37 bp) codes for the 5' untranslated region of the mRNA. The second exon codes for the signal peptide and the first 252 amino acids of the mature protein. Exons 3 and 4 are shorter and code for 90 and 48 amino acids, respectively. Exon 5 contains a short coding sequence (62 amino acids) and the 3'-untranslated region.

Plasma AGT is synthesized primarily in the liver and its expression is positively regulated by estrogens, glucocorticoids, thyroid hormones, and angiotensin II (Ang II) (Clauser et al., *Am. J. Hypertension* 2:403-410, 1989). Cleavage of the amino-terminal segment of AGT by renin releases a decapeptide prohormone, angiotensin-I, which is further processed to the active octapeptide angiotensin II by the dipeptidyl carboxypeptidase designated angiotensin-converting enzyme (ACE). Cleavage of AGT by renin is the rate-limiting step in the activation of the renin-angiotensin system.

Several epidemiological observations indicate a possible role of AGT in blood pressure regulation. A highly significant correlation between plasma AGT concentration and blood pressure has been observed in epidemiological studies (Walker et al., *J. Hypertension* 1:287-291, 1979). Interestingly, a number of allelic dimorphisms have been identified in the AGT gene. The frequency of at least two of them (174M and 235T) have been partially characterized and in certain populations shown to be significantly elevated in hypertensive subjects (Jeunemaitre et al., *Cell* 71:169-180, 1992). In addition, a specific polymorphism, 235T, has been suggested to be directly involved in coronary atherosclerosis (Ishigami et al., *Circulation* 91:951-4, 1995). Futhermore, the presence of A or G at position 1218 in the AGT regulatory region has been correlated with differences in *in vitro* transcriptional capacity for this gene (Inuoe et. al., *J. Clin. Invest.* 99:1786, 1997.

The human ACE gene is also a candidate as a marker for hypertension and myocardial infarction. ACE inhibitors constitute an important and effective therapeutic approach in the control of human hypertension (Sassaho et al. *Am. J. Med.* 83:227-235, 1987). In plasma and on the surface of endothelial cells, ACE converts the inactive angiotensin I molecule (Ang I) into active angiotensin II (Ang II) (Bottari et al., *Front. Neuroendocrinology* 14:123-171, 1993). Another ACE substrate is bradykinin, a potent vasodilator and inhibitor of smooth muscle cell proliferation, which is inactivated by ACE

(Ehlers et al., *Biochemistry* **28**:5311-5318, 1989; Erdos, E.G., *Hypertension* **16**:363-370, 1990; Johnston, C.I. *Drugs* (suppl. 1) **39**:21-31, 1990).

Levels of ACE are very stable within individuals, but differ greatly between individuals. Plasma ACE levels have been suggested to be genetically determined as a consequence of diallelic polymorphisms, situated within or close to the ACE gene. Prior to the present invention, no definitive association was demonstrated between polymorphisms and hypertension or blood pressure. However, a greater risk of myocardial infarction has been identified in a group of subjects with an ACE polymorphism designated ACE-DD (Cambien et al., *Nature* **359**:641-644, 1992), and a 12-fold greater risk of myocardial infarction has been identified in a subgroup of patients having a combination of the ACE polymorphism ACE-DD and one of the AGT polymorphisms (235T) described above (Kamitani et al., *Hypertension* **24**:381, 1994). Recently, six ACE polymorphisms were identified and characterized (Villard et al., *Am. J. Human Genet.* **58**:1268-1278, 1996).

The vasoconstrictive, cell growth-promoting and salt conserving actions of Ang II are mediated through binding to and activation of angiotensin receptors, of which at least two types have been cloned (AT1 and AT2). The type 1 Ang II receptor (AT1), a G-protein-coupled seven transmembrane domain protein, is widely distributed in the body and mediates almost all known Ang II effects (Fyhrquist et al., *J. Hum. Hypertension* **5**:519-524, 1995).

Several polymorphisms have been identified in the AT1 receptor gene. Initial studies suggest that at least one of them is more frequent in hypertensive subjects ($AT^{1166}C$)(Bonnardeaux et al., *Hypertension* **24**:63-69, 1994). This polymorphism, combined with the ACE-DD polymorphism, has been shown to correlate strongly with the risk of myocardial infarction (Tiret et al., *Lancet* **344**:910-913, 1994).

The high morbidity and mortality associated with cardiovascular disease demonstrate a need in the art for methods and compositions that allow the determination and/or prediction of the therapeutic regimen that will result in the most positive treatment outcome in a patient suffering from cardiovascular disease. This includes identification of individuals who are more or less susceptible to particular therapeutic regimens, including, e.g., particular drugs that are conventionally used to treat cardiovascular disease. There is also a need in the art for methods and compositions that allow the identification of individuals

having a predisposition to cardiovascular disease, such as, e.g., myocardial infarction, hypertension, atherosclerosis, and stroke, to facilitate early intervention and disease prevention.

Summary of the Invention

The present invention provides methods for assessing cardiovascular status in a human individual. Cardiovascular status is the physiological status of the cardiovascular system as reflected in one or more status markers. Status markers include without limitation clinical parameters such as, e.g., blood pressure or electrocardiographic profile, as well as diagnoses of cardiovascular status made by skilled medical practitioners, such as, e.g., acute myocardial infarction, silent myocardial infarction, stroke, and atherosclerosis. Also included in the evaluation of cardiovascular status are changes in status markers with time. The methods of the invention are carried out by the steps of:

(i) determining the sequence of one or more polymorphic positions within one or more of the genes encoding angiotensin converting enzyme (ACE), angiotensinogen (AGT), and type 1 angiotensin II receptor (AT1) in the individual to establish a polymorphic pattern for the individual; and

(ii) comparing the polymorphic pattern established in (i) with the polymorphic patterns of individuals exhibiting predetermined markers of cardiovascular status. The polymorphic pattern of the individual is, preferably, highly similar and, most preferably, identical to the polymorphic pattern of individuals who exhibit particular status markers, cardiovascular syndromes, and/or particular patterns of response to therapeutic interventions.

For example, a comparison of the polymorphic pattern of an individual with the polymorphic patterns of individuals exhibiting differing responses to a particular therapeutic intervention can be used to predict the degree of responsivity of the individual to such intervention. In a similar manner, the methods of the invention can be used to predict predisposition to different cardiovascular syndromes.

The invention also provides isolated nucleic acids encoding ACE, AGT, and AT1 in an individual, each of which comprises at least one polymorphic position. In preferred embodiments, the polymorphic position, either alone or in combination with other

polymorphic positions in the sequence of human ACE, AGT, or AT1, or in one or more other human genes, is predictive of a particular level of responsivity to a given treatment and/or indicates a predisposition to one or more clinical syndromes associated with cardiovascular disease.

The isolated nucleic acids according to the invention (which are described using the numbering indicated in Table 1 below) include without limitation:

(i) Nucleic acids encoding ACE having one or more polymorphic positions at the position in the regulatory region numbered 5106; positions in the coding region numbered 375, 582, 731, 1060, 2741, 3132, 3387, 3503, and 3906; and position 1451 as numbered in Genbank entry X62855. In preferred embodiments, the sequences at the polymorphic positions in the ACE regulatory region are one or more of 5106C and 5106T; and the sequences at the polymorphic positions in the coding region are one or more of 375A, 375C, 582C, 582T, 731A, 731G, 1060G, 1060A, 2741G, 2741T, 3132C, 3132T, 3387T, 3387C, 3503G, 3503C, 3906G, and 3906A. The invention also encompasses a nucleic acid encoding a deletion of nucleotides 1451-1783 as numbered in Genbank entry X62855.

(ii) Nucleic acids encoding AGT having one or more polymorphic positions at positions in the regulatory region numbered 395, 412, 432, 449, 692, 839, 1007, 1072, and 1204; positions in the coding region numbered 273, 912, 997, 1116, and 1174; and position 49 as numbered in Genbank entry M24688. In preferred embodiments, the sequences at the polymorphic positions in the AGT regulatory region are one or more of 395T, 395A, 412C, 412T, 432G, 432A, 449T, 449C, 692C, 692T, 839G, 839A, 1007G, 1007A, 1072G, 1072A, 1204C, and 1204A; the sequences at the polymorphic position in the coding region are one or more of 273C, 273T, 912C, 912T, 997G, 997C, 1116G, 1116A, 1174C and 1174A; and the sequence at position 49 in Genbank entry M24688 is either A or G.

(iii) Nucleic acids encoding AT1 having one or more polymorphic positions at positions in the regulatory region numbered 1427, 1756, 1853, 2046, 2354, 2355, and 2415; and the position in the coding region numbered 449. In preferred embodiments, the sequences at the polymorphic positions in the AT1 regulatory region are one or more of 1427A, 1427T, 1756T, 1756A, 1853T, 1853G, 2046T, 2046C, 2354A, 2354C, 2355G,

2355C, 2415A and 2415G; and the sequences at the polymorphic positions in the coding region are one or more of 449G, 449C, 678T, 678C, 1167A, 1167G, 1271A, and 1271C.

The invention also encompasses libraries of isolated nucleic acid sequences, wherein each sequence in the library comprises one or more polymorphic positions in the genes encoding human ACE, AGT, or AT1, including without limitation the polymorphic positions and sequences disclosed herein. Also provided are nucleic acid probes that hybridize specifically to the identified polymorphic positions; peptides and polypeptides comprising polymorphic positions; and polymorphism-specific antibodies, i.e., sequence-specific antibodies that bind differentially to polymorphic variants of ACE, AGT, or AT1 polypeptides and, preferably, can be used to identify particular polymorphic variants.

In yet another aspect, the invention provides kits for the determination of polymorphic patterns in an individual's ACE, AGT, and/or AT1 genes. The kits comprise a means for detecting polymorphic sequences, including without limitation oligonucleotide probes that hybridize at or adjacent to the polymorphic positions and polymorphism-specific antibodies.

In yet another aspect, the invention provides nucleic acid and polypeptide targets for use in screening methods to identify candidate cardiovascular drugs. Nucleic acid targets may be, e.g., DNA or RNA and are preferably at least about 10, and most preferably at least about 15, residues in length and comprise one or more polymorphic positions. Peptide targets are at least about 5 amino acids in length and may be as large or larger than full-length ACE, AGT, or AT1 polypeptides.

DETAILED DESCRIPTION OF THE INVENTION

All patents, patent applications, publications and other materials cited herein are hereby incorporated by reference in their entirety. In the case of inconsistencies, the present description, including definitions, is intended to control.

Definitions:

1. A "polymorphism" as used herein denotes a variation in the sequence of a gene in an individual. A "polymorphic position" is a predetermined nucleotide position within the sequence of a gene or a predetermined amino acid position in the sequence of a

polypeptide at which a polymorphism is located. An individual "homozygous" for a particular polymorphism is one in which both copies of the gene contain the same sequence at the polymorphic position. An individual "heterozygous" for a particular polymorphism is one in which the two copies of the gene contain different sequences at the polymorphic position.

2. A "polymorphism pattern" as used herein denotes a set of one or more polymorphisms, which may be contained in the sequence of a single gene or a plurality of genes. A polymorphism pattern may comprise nucleotide or amino acid polymorphisms.

3. "Nucleic acid" or "polynucleotide" as used herein refers to purine- and pyrimidine-containing polymers of any length, either polyribonucleotides or polydeoxyribonucleotides or mixed polyribo-polydeoxyribo nucleotides. Nucleic acids include without limitation single- and double-stranded molecules, i.e., DNA-DNA, DNA-RNA and RNA-RNA hybrids, as well as "protein nucleic acids" (PNA) formed by conjugating bases to an amino acid backbone. This also includes nucleic acids containing modified bases.

4. An "isolated" nucleic acid or polypeptide as used herein refers to a nucleic acid or polypeptide that is removed from its original environment (for example, its natural environment if it is naturally occurring). An isolated nucleic acid or polypeptide contains less than about 50%, preferably less than about 75%, and most preferably less than about 90%, of the cellular components with which it was originally associated.

5. A nucleic acid or polypeptide sequence that is "derived from" a designated sequence refers to a sequence that corresponds to a region of the designated sequence. For nucleic acid sequences, this encompasses sequences that are homologous or complementary to the sequence.

6. A "probe" refers to a nucleic acid or oligonucleotide that forms a hybrid structure with a sequence in a target nucleic acid due to complementarity of at least one sequence in the probe with a sequence in the target nucleic acid.

7. Nucleic acids are "hybridizable" to each other when at least one strand of nucleic acid can anneal to another nucleic acid strand under defined stringency conditions. Stringency of hybridization is determined, e.g., by a) the temperature at which hybridization and/or washing is performed, and b) the ionic strength and polarity (e.g., formamide) of the

hybridization and washing solutions, as well as other parameters. Hybridization requires that the two nucleic acids contain substantially complementary sequences; depending on the stringency of hybridization, however, mismatches may be tolerated. The appropriate stringency for hybridizing nucleic acids depends on the length of the nucleic acids and the degree of complementarity, variables well known in the art. For example, "high stringency" as used herein refers to hybridization and/or washing at 68°C in 0.2XSSC, at 42°C in 50% formamide, 4XSSC, or under conditions that afford levels of hybridization equivalent to those observed under either of these two conditions.

8. A "gene" for a particular protein as used herein refers to a contiguous nucleic acid sequence corresponding to a sequence present in a genome which comprises (i) a "coding region," which comprises exons (i.e., sequences encoding a polypeptide sequence or "protein-coding sequences"), introns, and sequences at the junction between exons and introns; and (ii) regulatory sequences, which flank the coding region at both 5' and 3' termini. For example, the "ACE gene" as used herein encompasses the regulatory and coding regions of the human gene encoding angiotensin converting enzyme. Similarly, the "AGT gene" encompasses regulatory and coding regions of the human gene encoding angiotensinogen and the "AT1 gene" encompasses regulatory and coding regions of the human gene encoding type I angiotensin II receptor. Typically, regulatory sequences according to the invention are located 5' (i.e., upstream) of the coding region segment. The reference sequences, obtained from Genbank, which were used in practicing the present invention are shown in Table 1.

Table 1

Abbreviation	Compared master sequence	Numbering according to sequence entry in GenBank
AGT Regulatory Region	X15323	X15323
AGT Coding Region	M24686 (exon 2) M24687 (exon 3) M24688 (exon 4) M24689 (exon 5) X62855 (intron 16)	Protein-coding sequences from exon 2-5 were spliced together as described in the GenBank entries. Nucleotide 1 is assigned to the first nucleotide of the initiator methionine codon. X62855
ACE Regulatory Region	X94359	X94359
ACE Coding Region	J04144	J04144 Nucleotide 1 is assigned to the first nucleotide of the initiator methionine codon.
AT1 Regulatory Region	U07144	U07144
AT1 Coding Region	S80239 (exon 3) S77410 (exon 5)	The protein-coding sequence of S80239 was spliced to position 288 of entry S77410. Nucleotide 1 is assigned to the first nucleotide of the initiator methionine codon in entry S80239.

The present inventors have surprisingly and unexpectedly discovered the existence of genetic polymorphisms within the human genes encoding ACE, AGT, and AT1 which, singly or in combination, can be used to assess cardiovascular status. In accordance with the invention, the polymorphic pattern of ACE, AGT, and/or AT1 sequences in an individual can predict the responsivity of the individual to particular therapeutic interventions and serve as an indicator of predisposition to various forms of cardiovascular disease. The invention provides methods for assessing cardiovascular status by detecting polymorphic

patterns in an individual. The present invention also provides isolated nucleic acids derived from the ACE, AGT, and AT1 genes which comprise these polymorphisms, including probes which hybridize specifically to polymorphic positions; isolated polypeptides and peptides comprising polymorphic residues; and antibodies which specifically recognize ACE, AGT, or AT1 polypeptides containing one or more polymorphic amino acids.

Methods for Assessing Cardiovascular Status

The present invention provides diagnostic methods for assessing cardiovascular status in a human individual. Cardiovascular status as used herein refers to the physiological status of an individual's cardiovascular system as reflected in one or more markers or indicators. Status markers include without limitation clinical measurements such as, e.g., blood pressure, electrocardiographic profile, and differentiated blood flow analysis. Status markers according to the invention include diagnoses of one or more cardiovascular syndromes, such as, e.g., hypertension, acute myocardial infarction, silent myocardial infarction, stroke, and atherosclerosis. It will be understood that a diagnosis of a cardiovascular syndrome made by a medical practitioner encompasses clinical measurements and medical judgement. Status markers according to the invention are assessed using conventional methods well known in the art. Also included in the evaluation of cardiovascular status are quantitative or qualitative changes in status markers with time, such as would be used, e.g., in the determination of an individual's response to a particular therapeutic regimen.

The methods are carried out by the steps of:

(i) determining the sequence of one or more polymorphic positions within one or more of the genes encoding angiotensin coverting enzyme (ACE), angiotensinogen (AGT), or type 1 angiotensin II receptor (AT1) in the individual to establish a polymorphic pattern for the individual; and

(ii) comparing the polymorphic pattern established in (i) with the polymorphic patterns of humans exhibiting different markers of cardiovascular status. The polymorphic pattern of the individual is, preferably, highly similar and, most preferably, identical to the polymorphic pattern of individuals who exhibit particular status markers, cardiovascular

syndromes, and/or particular patterns of response to therapeutic interventions. Polymorphic patterns may also include polymorphic positions in other genes which are shown, in combination with one or more polymorphic positions in ACE, AGT, or AT1, to correlate with the presence of particular status markers.

In one embodiment, the method involves comparing an individual's polymorphic pattern with polymorphic patterns of individuals who have been shown to respond positively or negatively to a particular therapeutic regimen. Therapeutic regimen as used herein refers to treatments aimed at the elimination or amelioration of symptoms and events associated cardiovascular disease. Such treatments include without limitation one or more of alteration in diet, lifestyle, and exercise regimen; invasive and noninvasive surgical techniques such as atherectomy, angioplasty, and coronary bypass surgery; and pharmaceutical interventions, such as administration of ACE inhibitors, angiotensin II receptor antagonists, diuretics, alpha-adrenoreceptor antagonists, cardiac glycosides, phosphodiesterase inhibitors, beta-adrenoreceptor antagonists, calcium channel blockers, HMG-CoA reductase inhibitors, imidazoline receptor blockers, endothelin receptor blockers, and organic nitrites. Interventions with pharmaceutical agents not yet known whose activity correlates with particular polymorphic patterns associated with cardiovascular disease are also encompassed. The present inventors have discovered that particular polymorphic patterns correlate with an individual's responsivity to ACE inhibitors (see, e.g., Example 3 below). It is contemplated, for example, that patients who are candidates for a particular therapeutic regimen will be screened for polymorphic patterns that correlate with responsivity to that particular regimen.

In a preferred embodiment, the presence or absence in an individual of a polymorphic pattern comprising ACE 2193 A/G, AGR 1072 G/G, and AT1 1167 A/A (see below) is determined to identify an individual's responsivity to ACE inhibitors.

In another embodiment, the method involves comparing an individual's polymorphic pattern with polymorphic patterns of individuals who exhibit or have exhibited one or more markers of cardiovascular disease, such as, e.g., high blood pressure, abnormal electrocardiographic profile, myocardial infarction, stroke, or atherosclerosis (see, e.g., Example 2 below).

In practicing the methods of the invention, an individual's polymorphic pattern can be established by obtaining DNA from the individual and determining the sequence at predetermined polymorphic positions in ACE, AGT, and AT1 such as those described above.

The DNA may be obtained from any cell source. Non-limiting examples of cell sources available in clinical practice include blood cells, buccal cells, cervicovaginal cells, epithelial cells from urine, fetal cells, or any cells present in tissue obtained by biopsy. Cells may also be obtained from body fluids, including without limitation blood, saliva, sweat, urine, cerebrospinal fluid, feces, and tissue exudates at the site of infection or inflammation. DNA is extracted from the cell source or body fluid using any of the numerous methods that are standard in the art. It will be understood that the particular method used to extract DNA will depend on the nature of the source.

Determination of the sequence of the extracted DNA at polymorphic positions in ACE, AGT, and/or AT1 genes is achieved by any means known in the art, including but not limited to direct sequencing, hybridization with allele-specific oligonucleotides, allele-specific PCR, ligase-PCR, HOT cleavage, denaturing gradient gel electrophoresis (DDGE), and single-stranded conformational polymorphism (SSCP). Direct sequencing may be accomplished by any method, including without limitation chemical sequencing, using the Maxam-Gilbert method; by enzymatic sequencing, using the Sanger method; mass spectrometry sequencing; and sequencing using a chip-based technology. See, e.g., Little et al., *Genet. Anal.* **6**:151, 1996. Preferably, DNA from a subject is first subjected to amplification by polymerase chain reaction (PCR) using specific amplification primers.

In an alternate embodiment, biopsy tissue is obtained from a subject. Antibodies that are capable of distinguishing between different polymorphic forms ACE, AGT, and/or AT1 are then applied to samples of the tissue to determine the presence or absence of a polymorphic form specified by the antibody. The antibodies may be polyclonal or monoclonal, preferably monoclonal. Measurement of specific antibody binding to cells may be accomplished by any known method e.g. quantitative flow cytometry, or enzyme-linked or fluorescence-linked immunoassay. The presence or absence of a particular polymorphism or polymorphic pattern, and its allelic distribution (i.e., homozygosity vs.

heterozygosity) is determined by comparing the values obtained from a patient with norms established from populations of patients having known polymorphic patterns.

In an alternate embodiment, RNA is isolated from biopsy tissue using standard methods well known to those of ordinary skill in the art such as guanidium thiocyanate-phenol-chloroform extraction (Chomocyznski et al., 1987, *Anal. Biochem.*, **162**:156.) The isolated RNA is then subjected to coupled reverse transcription and amplification by polymerase chain reaction (RT-PCR), using specific oligonucleotide primers. Conditions for primer annealing are chosen to ensure specific reverse transcription and amplification; thus, the appearance of an amplification product is diagnostic of the presence of particular alleles. In another embodiment, RNA is reverse-transcribed and amplified, after which the amplified sequences are identified by, e.g., direct sequencing.

In practicing the present invention, the distribution of polymorphic patterns in a large number of individuals exhibiting particular markers of cardiovascular status is determined by any of the methods described above, and compared with the distribution of polymorphic patterns in patients that have been matched for age, ethnic origin, and/or any other statistically or medically relevant parameters, who exhibit quantitatively or qualitatively different status markers. Correlations are achieved using any method known in the art, including nominal logistic regression or standard least-squares regression analysis. In this manner, it is possible to establish statistically significant correlations between particular polymorphic patterns and particular cardiovascular statuses. It is further possible to establish statistically significant correlations between particular polymorphic patterns and changes in cardiovascular status such as, would result, e.g., from particular treatment regimens. In this manner, it is possible to correlate polymorphic patterns with responsivity to particular treatments.

Polymorphic Positions in Genes Encoding ACE, AGT, and AT1

Polymorphic positions in the genes encoding ACE, AGT, and AT1 which are encompassed by the invention are identified by determining the DNA sequence of all or part of the ACE, AGT, and/or AT1 genes in a multiplicity of individuals in a population. DNA

sequence determination may be achieved using any conventional method, including, e.g., chemical or enzymatic sequencing.

The polymorphic positions of the invention include without limitation those listed below, whose numbering corresponds to the Genbank sequences listed in Table 1.

(i) ACE: positions in the regulatory region (designated ACR) numbered 5106, 5349, and 5496; positions in the coding region (designated ACE) numbered 375, 582, 731, 1060, 1215, 2193, 2328, 2741, 3132, 3387, 3503, and 3906; and position 1451 as numbered in Genbank entry X62855.

(ii) AGT: positions in the regulatory region (designated AGR) numbered 395, 412, 432, 449, 692, 839, 1007, 1072, 1204, and 1218; positions in the coding region (designated AGT) numbered 273, 620, 803, 912, 997, 1116, and 1174; and position 49 as numbered in Genbank entry M24688.

(iii) AT1: positions in the regulatory region (designated ATR) numbered 1427, 1756, 1853, 2046, 2354, 2355, and 2415; and positions in the coding region (designated AT1) numbered 449, 678, 1167, and 1271.

In preferred embodiments, the sequence at each of the above polymorphic positions is one of:

(i) ACE Regulatory Region: 5106C, 5106T, 5349A, 5349T, 5496T, and 5496C;

(ii) ACE Coding Region: 375A, 375C, 582C, 582T, 731A, 731G, 1060G, 1060A, 1215C, 1215T, 2193G, 2193A, 2328A, 2328G, 2741G, 2741T, 3132C, 3132T, 3387T, 3387C, 3503G, 3503C, 3906G, and 3906A; and a deletion of nucleotides 1451-1783 as numbered in Genbank entry X62855;

(iii) AGT Regulatory Region: 395T, 395A, 412C, 412T, 432G, 432A, 449T, 449C, 692C, 692T, 839G, 839A, 1007G, 1007A, 1072G, 1072A, 1204C, 1204A, 1218A, 1218G;

(iv) AGT Coding Region: 273C, 273T, 620C, 620T, 803T, 803C, 912C, 912T, 997G, 997C, 1116G, 1116A, 1174C, and 1174A; and A or G at position 49 in Genbank entry M24688;

(v) AT1 Regulatory Region: 1427A, 1427T, 1756T, 1756A, 1853T, 1853G, 2046T, 2046C, 2354A, 2354C, 2355G, 2355C, 2415A and 2415G; and

(vi) AT1 Coding Region: 449G, 449C, 678T, 678C, 1167A, 1167G, 1271A, and 1271C.

An individual may be homozygous or heterozygous for a particular polymorphic position (see, e.g., Table 6 below).

Non-limiting examples of polymorphic patterns comprising one or more polymorphism in ACE, AGT, and/or AT1 genes according to the invention include the following, which were correlated with an increased incidence of clinical signs of cardiovascular disease:

ACR 5349 A/T, AGR 1218 A; ACR 5496 C, AGR 1204 A/C; ACR 5496 C/T, AGR 1218 A, AGT 620 C/T; ACE 2193 A, AGR 1204 C, ACE 2328 G; ACE 2193 A, AGR 1204 A/C; ACE 3387 T, AGR 1218 A; ACE 3387 T, AGT 620 C/T; AGR 1204 A/C, AT1 678 C/T; AGR 1204 A/C, AT1 1271 A/C; ACE 1215 C, AGR 1204 A/C; AGR 1204 A/C, AT1 1167 A, ACE 3906 A/G; AGR 1204 A, AGT 620 C, AT1 1271 A, AT1 1167 A, AGR 395 A/T; AGR 1204 A/C, AGT 620 C/T, AT1 1271 A/C, AT1 1167 A, AGR 395 T; AGR 1204 A/C, AGT 620 C/T, AT1 1271 A/C, AT1 1167 A/G, AGR 395 T; AGR 1204 A, AT1 678 C, AT1 1167 A, AGR 395 A/T; AGR 1204 A/C, AT1 678 C/T, AT1 1167 A, AGR 395 T; AGT 620 C/T, AT1 1271 A/C, AT1 1167 A, AGR 395 T; AGT 620 C/T, AT1 1271 A/C, AT1 1167 A/G, AGR 395 T; AGT 620 C, AT1 1271 A, AT1 1167 A, AGR 395 A/T; AGT 620 C, AT1 678 A, AT1 1167 A, AGR 395 A/T; AGT 620 C/T, AT1 678 C/T; AT1 1167 A, AGR 395 T; ACE 2193 A, AGR 1218 A, AGT 803 A; ACE 2193 A, AGT 620 C/T; ACE 2328 G, AGT 620 C/T; ACE 3387 T, AGR 1204 A/C; ACE 2193 A, ACE 2328 G, AGR 1204 C; and ACE 2193 A/G, AGR 1072 G/G, AT1 1167 A/A.

Isolated Polymorphic Nucleic Acids, Probes, and Vectors

The present invention provides isolated nucleic acids comprising the polymorphic positions described above for the human ACE, AGT, and AT1 genes; vectors comprising the nucleic acids; and transformed host cells comprising the vectors. The invention also provides probes which are useful for detecting these polymorphisms.

In practicing the present invention, many conventional techniques in molecular biology, microbiology, and recombinant DNA, are used. Such techniques are well known and are explained fully in, for example, Sambrook et al., 1989, *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; *DNA Cloning: A Practical Approach*, Volumes I and II, 1985 (D.N. Glover ed.); *Oligonucleotide Synthesis,* 1984, (M.L. Gait ed.); *Nucleic Acid Hybridization*, 1985, (Hames and Higgins); Ausubel et al., *Current Protocols in Molecular Biology*, 1997, (John Wiley and Sons); and *Methods in Enzymology* Vol. 154 and Vol. 155 (Wu and Grossman, and Wu, eds., respectively).

Insertion of nucleic acids (typically DNAs) comprising the sequences of the present invention into a vector is easily accomplished when the termini of both the DNAs and the vector comprise compatible restriction sites. If this cannot be done, it may be necessary to modify the termini of the DNAs and/or vector by digesting back single-stranded DNA overhangs generated by restriction endonuclease cleavage to produce blunt ends, or to achieve the same result by filling in the single-stranded termini with an appropriate DNA polymerase.

Alternatively, any site desired may be produced, e.g., by ligating nucleotide sequences (linkers) onto the termini. Such linkers may comprise specific oligonucleotide sequences that define desired restriction sites. Restriction sites can also be generated by the use of the polymerase chain reaction (PCR). See, e.g., Saiki *et al.*, 1988, *Science* **239**:48. The cleaved vector and the DNA fragments may also be modified if required by homopolymeric tailing.

The nucleic acids may be isolated directly from cells or may be chemically synthesized using known methods. Alternatively, the polymerase chain reaction (PCR) method can be used to produce the nucleic acids of the invention, using either chemically synthesized strands or genomic material as templates. Primers used for PCR can be synthesized using the sequence information provided herein and can further be designed to introduce appropriate new restriction sites, if desirable, to facilitate incorporation into a given vector for recombinant expression.

The nucleic acids of the present invention may be flanked by native ACE, AGT, or AT1 gene sequences, or may be associated with heterologous sequences, including

promoters, enhancers, response elements, signal sequences, polyadenylation sequences, introns, 5'- and 3'- noncoding regions, and the like. The nucleic acids may also be modified by many means known in the art. Non-limiting examples of such modifications include methylation, "caps", substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoroamidates, carbamates, etc.) and with charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.). Nucleic acids may contain one or more additional covalently linked moieties, such as, for example, proteins (e.g., nucleases, toxins, antibodies, signal peptides, poly-L-lysine, etc.), intercalators (e.g., acridine, psoralen, etc.), chelators (e.g., metals, radioactive metals, iron, oxidative metals, etc.), and alkylators. PNAs are also included. The nucleic acid may be derivatized by formation of a methyl or ethyl phosphotriester or an alkyl phosphoramidate linkage. Furthermore, the nucleic acid sequences of the present invention may also be modified with a label capable of providing a detectable signal, either directly or indirectly. Exemplary labels include radioisotopes, fluorescent molecules, biotin, and the like.

The invention also provides nucleic acid vectors comprising the disclosed ACE, AGT, and AT1-derived gene sequences or derivatives or fragments thereof. A large number of vectors, including plasmid and fungal vectors, have been described for replication and/or expression in a variety of eukaryotic and prokaryotic hosts, and may be used for gene therapy as well as for simple cloning or protein expression. Non-limiting examples of suitable vectors include without limitation pUC plasmids, pET plasmids (Novagen, Inc., Madison, WI), or pRSET or pREP (Invitrogen, San Diego, CA), and many appropriate host cells, using methods disclosed or cited herein or otherwise known to those skilled in the relevant art. The particular choice of vector/host is not critical to the practice of the invention.

Suitable host cells may be transformed/transfected/infected as appropriate by any suitable method including electroporation, $CaCl_2$ mediated DNA uptake, fungal or viral infection, microinjection, microprojectile, or other established methods. Appropriate host cells included bacteria, archebacteria, fungi, especially yeast, and plant and animal cells, especially mammalian cells. A large number of transcription initiation and termination regulatory regions have been isolated and shown to be effective in the transcription and

translation of heterologous proteins in the various hosts. Examples of these regions, methods of isolation, manner of manipulation, etc. are known in the art. Under appropriate expression conditions, host cells can be used as a source of recombinantly produced ACE-, AGT-, or AT1-derived peptides and polypeptides.

Nucleic acids encoding ACE-, AGT-, or AT1-derived gene sequences may also be introduced into cells by recombination events. For example, such a sequence can be introduced into a cell and thereby effect homologous recombination at the site of an endogenous gene or a sequence with substantial identity to the gene. Other recombination-based methods such as nonhomologous recombinations or deletion of endogenous genes by homologous recombination may also be used.

The nucleic acids of the present invention find use as probes for the detection of genetic polymorphisms and as templates for the recombinant production of normal or variant ACE-, AGT-, or AT1-derived peptides or polypeptides.

Probes in accordance with the present invention comprise without limitation isolated nucleic acids of about 10 - 100 bp, preferably 15-75 bp and most preferably 17-25 bp in length, which hybridize at high stringency to one or more of the ACE, AGT, or AT1 gene-derived polymorphic sequences disclosed herein or to a sequence immediately adjacent to a polymorphic position. Furthermore, in some embodiments a full-length gene sequence may be used as a probe. In one series of embodiments, the probes span the polymorphic positions in the ACE, AGT, or AT1 genes disclosed above. In another series of embodiments, the probes correspond to sequences immediately adjacent to the polymorphic positions.

Polymorphic ACE, AGT, and AT1 Polypeptides and Polymorphism-Specific Antibodies

The present invention encompasses isolated peptides and polypeptides encoding ACE, AGT, and AT1 comprising polymorphic positions disclosed above. In one preferred embodiment, the peptides and polypeptides are useful screening targets to identify cardiovascular drugs. In another preferred embodiments, the peptides and polypeptides are capable of eliciting antibodies in a suitable host animal that react specifically with a

polypeptide comprising the polymorphic position and distinguish it from other polypeptides having a different sequence at that position.

Polypeptides according to the invention are preferably at least five or more residues in length, preferably at least fifteen residues. Methods for obtaining these polypeptides are described below. Many conventional techniques in protein biochemistry and immunology are used. Such techniques are well known and are explained in *Immunochemical Methods in Cell and Molecular Biology*, 1987 (Mayer and Waler, eds; Academic Press, London); Scopes, 1987, *Protein Purification: Principles and Practice*, Second Edition (Springer-Verlag, N.Y.) and *Handbook of Experimental Immunology*, 1986, Volumes I-IV (Weir and Blackwell eds.).

Nucleic acids comprising protein-coding sequences can be used to direct the recombinant expression of ACE-, AGT, or AT1-derived polypeptides in intact cells or in cell-free translation systems. The known genetic code, tailored if desired for more efficient expression in a given host organism, can be used to synthesize oligonucleotides encoding the desired amino acid sequences. The polypeptides may be isolated from human cells, or from heterologous organisms or cells (including, but not limited to, bacteria, fungi, insect, plant, and mammalian cells) into which an appropriate protein-coding sequence has been introduced and expressed. Furthermore, the polypeptides may be part of recombinant fusion proteins.

Peptides and polypeptides may be chemically synthesized by commercially available automated procedures, including, without limitation, exclusive solid phase synthesis, partial solid phase methods, fragment condensation or classical solution synthesis. The polypeptides are preferably prepared by solid phase peptide synthesis as described by Merrifield, 1963, *J. Am. Chem. Soc.* **85**:2149.

Methods for polypeptide purification are well-known in the art, including, without limitation, preparative disc-gel electrophoresis, isoelectric focusing, HPLC, reversed-phase HPLC, gel filtration, ion exchange and partition chromatography, and countercurrent distribution. For some purposes, it is preferable to produce the polypeptide in a recombinant system in which the protein contains an additional sequence tag that facilitates purification, such as, but not limited to, a polyhistidine sequence. The polypeptide can then be purified from a crude lysate of the host cell by chromatography on an appropriate solid-phase matrix.

Alternatively, antibodies produced against ACE, AGT, or AT1, or against peptides derived therefrom, can be used as purification reagents. Other purification methods are possible.

The present invention also encompasses derivatives and homologues of the polypeptides. For some purposes, nucleic acid sequences encoding the peptides may be altered by substitutions, additions, or deletions that provide for functionally equivalent molecules, i.e., function-conservative variants. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of similar properties, such as, for example, positively charged amino acids (arginine, lysine, and histidine); negatively charged amino acids (aspartate and glutamate); polar neutral amino acids; and non-polar amino acids.

The isolated polypeptides may be modified by, for example, phosphorylation, sulfation, acylation, or other protein modifications. They may also be modified with a label capable of providing a detectable signal, either directly or indirectly, including, but not limited to, radioisotopes and fluorescent compounds.

The present invention also encompasses antibodies that specifically recognize the polymorphic positions of the invention and distinguish a peptide or polypeptide containing a particular polymorphism from one that contains a different sequence at that position. Such polymorphic position-specific antibodies according to the present invention include polyclonal and monoclonal antibodies. The antibodies may be elicited in an animal host by immunization with ACE, AGT, or AT1-derived immunogenic components or may be formed by *in vitro* immunization of immune cells. The immunogenic components used to elicit the antibodies may be isolated from human cells or produced in recombinant systems. The antibodies may also be produced in recombinant systems programmed with appropriate antibody-encoding DNA. Alternatively, the antibodies may be constructed by biochemical reconstitution of purified heavy and light chains. The antibodies include hybrid antibodies (i.e., containing two sets of heavy chain/light chain combinations, each of which recognizes a different antigen), chimeric antibodies (i.e., in which either the heavy chains, light chains, or both, are fusion proteins), and univalent antibodies (i.e., comprised of a heavy chain/light chain complex bound to the constant region of a second heavy chain). Also included are Fab fragments, including Fab' and $F(ab)_2$ fragments of antibodies. Methods for the production

of all of the above types of antibodies and derivatives are well-known in the art and are discussed in more detail below. For example, techniques for producing and processing polyclonal antisera are disclosed in Mayer and Walker, 1987, *Immunochemical Methods in Cell and Molecular Biology,* (Academic Press, London). The general methodology for making monoclonal antibodies by hybridomas is well known. Immortal antibody-producing cell lines can be created by cell fusion, and also by other techniques such as direct transformation of B lymphocytes with oncogenic DNA, or transfection with Epstein-Barr virus. See, e.g., Schreier *et al.*, 1980, *Hybridoma Techniques*; U.S. Patent Nos. 4,341,761; 4,399,121; 4,427,783; 4,444,887; 4,466,917; 4,472,500; 4,491,632; and 4,493,890. Panels of monoclonal antibodies produced against ACE, AGT, or AT1-derived epitopes can be screened for various properties; i.e. for isotype, epitope affinity, etc.

The antibodies of this invention can be purified by standard methods, including but not limited to preparative disc-gel electrophoresis, isoelectric focusing, HPLC, reversed-phase HPLC, gel filtration, ion exchange and partition chromatography, and countercurrent distribution. Purification methods for antibodies are disclosed, e.g., in *The Art of Antibody Purification*, 1989, Amicon Division, W.R. Grace & Co. General protein purification methods are described in *Protein Purification: Principles and Practice*, R.K. Scopes, Ed., 1987, Springer-Verlag, New York, NY.

Methods for determining the immunogenic capability of the disclosed sequences and the characteristics of the resulting sequence-specific antibodies and immune cells are well-known in the art. For example, antibodies elicited in response to a peptide comprising a particular polymorphic sequence can be tested for their ability to specifically recognize that polymorphic sequence, i.e., to bind differentially to a peptide or polypeptide comprising the polymorphic sequence and thus distinguish it from a similar peptide or polypeptide containing a different sequence at the same position.

Diagnostic Methods and Kits

The present invention provides kits for the determination of the sequence at polymorphic positions within the ACE, AGT, and AT1 genes in an individual. The kits comprise a means for determining the sequence at one or more polymorphic positions, and

may optionally include data for analysis of polymorphic patterns. The means for sequence determination may comprise suitable nucleic acid-based and immunological reagents (see below). Preferably, the kits also comprise suitable buffers, control reagents where appropriate, and directions for determining the sequence at a polymorphic position. The kits may also comprise data for correlation of particular polymorphic patterns with desirable treatment regimens or other indicators.

Nucleic-acid-based diagnostic methods and kits:

The invention provides nucleic acid-based methods for detecting polymorphic patterns in a biological sample. The sequence at particular polymorphic positions in the genes encoding ACE, AGT, and/or AT1 is determined using any suitable means known in the art, including without limitation hybridization with polymorphism-specific probes and direct sequencing.

The present invention also provides kits suitable for nucleic acid-based diagnostic applications. In one embodiment, diagnostic kits include the following components:

(i) *Probe DNA:* The probe DNA may be pre-labelled; alternatively, the probe DNA may be unlabelled and the ingredients for labelling may be included in the kit in separate containers; and

(ii) *Hybridization reagents:* The kit may also contain other suitably packaged reagents and materials needed for the particular hybridization protocol, including solid-phase matrices, if applicable, and standards.

In another embodiment, diagnostic kits include:

(i) *Sequence determination primers:* Sequencing primers may be pre-labelled or may contain an affinity purification or attachment moiety; and

(ii) *Sequence determination reagents:* The kit may also contain other suitably packaged reagents and materials needed for the particular sequencing protocol. In one preferred embodiment, the kit comprises a panel of sequencing primers, whose sequences correspond to sequences adjacent to the following polymorphic positions: ACE 2193 A/G, AGR 1072 G/G, AT1 1167 A/A; as well as a means for detecting the presence of each polymorphic sequence.

Antibody-based diagnostic methods and kits:

The invention also provides antibody-based methods for detecting polymorphic patterns in a biological sample. The methods comprise the steps of: (i) contacting a sample with one or more antibody preparations, wherein each of the antibody preparations is specific for a particular polymorphic form of either ACE, AGT, or AT1, under conditions in which a stable antigen-antibody complex can form between the antibody and antigenic components in the sample; and (ii) detecting any antigen-antibody complex formed in step (i) using any suitable means known in the art, wherein the detection of a complex indicates the presence of the particular polymorphic form in the sample.

Typically, immunoassays use either a labelled antibody or a labelled antigenic component (e.g., that competes with the antigen in the sample for binding to the antibody). Suitable labels include without limitation enzyme-based, fluorescent, chemiluminescent, radioactive, or dye molecules. Assays that amplify the signals from the probe are also known, such as, for example, those that utilize biotin and avidin, and enzyme-labelled immunoassays, such as ELISA assays.

The present invention also provides kits suitable for antibody-based diagnostic applications. Diagnostic kits typically include one or more of the following components:

(i) *Polymorphism-specific antibodies:* The antibodies may be pre-labelled; alternatively, the antibody may be unlabelled and the ingredients for labelling may be included in the kit in separate containers, or a secondary, labelled antibody is provided; and

(ii) *Reaction components:* The kit may also contain other suitably packaged reagents and materials needed for the particular immunoassay protocol, including solid-phase matrices, if applicable, and standards.

The kits referred to above may include instructions for conducting the test. Furthermore, in preferred embodiments, the diagnostic kits are adaptable to high-throughput and/or automated operation.

Drug Targets and Screening Methods

According to the present invention, nucleotide sequences derived from genes encoding ACE, AGT, and AT1 and peptide sequences derived from ACE, AGT, and AT1

polypeptides, particularly those that contain one or more polymorphic sequences, comprise useful targets to identify cardiovascular drugs, i.e., compounds that are effective in treating one or more clinical symptoms of cardiovascular disease.

Drug targets include without limitation (i) isolated nucleic acids derived from the genes encoding ACE, AGT, and AT1, and (ii) isolated peptides and polypeptides derived from ACE, AGT, and AT1 polypeptides, each of which comprises one or more polymorphic positions.

In vitro screening methods:

In one series of embodiments, an isolated nucleic acid comprising one or more polymorphic positions is tested *in vitro* for its ability to bind test compounds in a sequence-specific manner. The methods comprise:

(i) providing a first nucleic acid containing a particular sequence at a polymorphic position and a second nucleic acid whose sequence is identical to that of the first nucleic acid except for a different sequence at the same polymorphic position;

(ii) contacting the nucleic acids with a multiplicity of test compounds under conditions appropriate for binding; and

(iii) identifying those compounds that bind selectively to either the first or second nucleic acid sequence.

Selective binding as used herein refers to any measurable difference in any parameter of binding, such as, e.g., binding affinity, binding capacity, etc.

In another series of embodiments, an isolated peptide or polypeptide comprising one or more polymorphic positions is tested *in vitro* for its ability to bind test compounds in a sequence-specific manner. The screening methods involve:

(i) providing a first peptide or polypeptide containing a particular sequence at a polymorphic position and a second peptide or polypeptide whose sequence is identical to the first peptide or polypeptide except for a different sequence at the same polymorphic position;

(ii) contacting the polypeptides with a multiplicity of test compounds under conditions appropriate for binding; and

(iii) identifying those compounds that bind selectively to one of the nucleic acid sequences.

In preferred embodiments, high-throughput screening protocols are used to survey a large number of test compounds for their ability to bind the genes or peptides disclosed above in a sequence-specific manner.

Test compounds are screened from large libraries of synthetic or natural compounds. Numerous means are currently used for random and directed synthesis of saccharide, peptide, and nucleic acid based compounds. Synthetic compound libraries are commercially available from Maybridge Chemical Co. (Trevillet, Cornwall, UK), Comgenex (Princeton, NJ), Brandon Associates (Merrimack, NH), and Microsource (New Milford, CT). A rare chemical library is available from Aldrich (Milwaukee, WI). Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available from e.g. Pan Laboratories (Bothell, WA) or MycoSearch (NC), or are readily producible. Additionally, natural and synthetically produced libraries and compounds are readily modified through conventional chemical, physical, and biochemical means.

In vivo screening methods:

Intact cells or whole animals expressing polymorphic variants of genes encoding ACE, AGT, and/or AT1 can be used in screening methods to identify candidate cardiovascular drugs.

In one series of embodiments, a permanent cell line is established from an individual exhibiting a particular polymorphic pattern. Alternatively, cells (including without limitation mammalian, insect, yeast, or bacterial cells) are programmed to express a gene comprising one or more polymorphic sequences by introduction of appropriate DNA. Identification of candidate compounds can be achieved using any suitable assay, including without limitation (i) assays that measure selective binding of test compounds to particular polymorphic variants of ACE, AGT, or AT1; (ii) assays that measure the ability of a test compound to modify (i.e., inhibit or enhance) a measurable activity or function of ACE, AGT, or AT1; and (iii) assays that measure the ability of a compound to modify (i.e., inhibit or enhance) the transcriptional activity of sequences derived from the promoter (i.e., regulatory) regions of ACE, AGT, or AT1 genes.

In another series of embodiments, transgenic animals are created in which (i) one or more human ACE, AGT, or AT1 genes having different sequences at particular polymorphic positions are stably inserted into the genome of the transgenic animal; and/or (ii) the endogenous ACE, AGT, and/or AT1 genes are inactivated and replaced with human ACE, AGT, and/or AT1 genes having different sequences at particular polymorphic positions. See, e.g., Coffman, *Semin. Nephrol.* 17:404, 1997; Esther et al., *Lab. Invest.* 74:953, 1996; Murakami et al., *Blood Press. Suppl.* 2:36, 1996. Such animals can be treated with candidate compounds and monitored for one or more clinical markers of cardiovascular status.

The following are intended as non-limiting examples of the invention.

Example 1: Methods for Identification of Polymorphic Positions in Human Genes Encoding ACE, AGT, and AT1

The following studies were performed to identify polymorphic residues within the genes encoding human ACE, AGT, and AT1.

DNA samples were obtained from 277 individuals. The individuals were Caucasian males born in Uppsala, Sweden between 1920 and 1924. Individuals were selected for the test population based on their medical history, i.e., they were either (i) healthy, with no signs of cardiovascular disease (100); or (ii) had suffered one of acute myocardial infarction (68), silent myocardial infarction (34), stroke (18), stroke and acute myocardial infarction (19), or high blood pressure at age 50 (39). DNA samples were obtained from each individual.

DNA sequence analysis was carried out by: (i) amplifying short fragments of each of the ACE, AGT, and AT1 genes using polymerase chain reaction (PCR) and (ii) sequencing the amplified fragments. The sequences obtained from each individual were then compared with known ACE, AGT, and AT1 genomic sequences (see Table 1).

(i) Amplification: PCR reactions employed the primers shown in Table 2 below.

Table 2

Name	Sequence	Modification *)	Nucleotides	Numbering according to **)
ACE/79RB	5'-TGCGTGCTTCAGAAGTCC-3'	B	158-175	i+20: 1-175
ACE/82RB	5'-CCAGGGAGGTGAAGAAATC-3'	B	35-53	e20, J04144
ACE/84FT	5'-AGCCAGGCAGTAATGACCT-3'	T	1-19	i-19: 1-218
ACE/94FB	5'-GCCCACTGTTCCCTTATG-3'	B	1-18	i-21: 1-76
ACE/95RB	5'-TGCCCTGACTGACAGAGC-3'	B	105-122	i+23: 1-122
ACE/96RT	5'-GCCCTGGTGTGCCTGT-3'	T	1-16	i-22: 1-65
ACE/107F	5'-TGCCTGGATATGTGTTGC-3'	-	1-18	i-15: 1-225
ACE/107FB	5'-TGCCTGGATATGTGTTGC-3'	B	1-18	i-15: 1-225
ACE/108RB	5'-GCCCTCGCCTCTCACT-3'	B	23-38	i+16: 1-38
ACE/111RT	5'-TCCCCTCTCCCTGTACCT-3'	T	17-34	i+15: 1-34
ACE/114RB	5'-GTGCTGGGGTAGGGTAGA-3'	B	101-118	i+7: 1-118
ACE/118FT	5'-TCCCCCTGACCTGGCT-3'	T	221-236	i-7: 1-253
ACE/119FB	5'-GGGGCACCGTGATGTT-3'	B	1-16	i-4: 1-120
ACE/119FT	5'-GGGGCACCGTGATGTT-3'	T	1-16	i-4: 1-120
ACE/120RB	5'-GCCAGAGCCTTTGGTTT-3'	B	230-246	i+5: 1-246
ACE/122FB	5'-TGGAAGAGCCGACTTACAG-3'	B	1-19	i-5: 1-78
ACE/123RB	5'-TCCCAGAGGCAAAGAGG-3'	B	225-241	i+4: 1-241
ACE/130F	5'-GTTTCTACTGCGGCTTCAT-3'	-	1-19	i-8: 1-131
ACE/130FB	5'-GTTTCTACTGCGGCTTCAT-3'	B	1-19	i-8: 1-131
ACE/134RB	5'-TCCTGGAAGAGGGAGTTTC-3'	B	148-166	i+9: 1-166

Name	Sequence	Modification *)	Nucleotides	Numbering according to **)
ACE/145F	5'-GCAGGATGAGAGCAACAAC-3'	-	1-18	i-7: 1-253
ACE/146F	5'-CTGGAGACCACTCCCATCCTTTCT-3'	-	1-24	i-17: 1-454
ACE/147R	5'-GATGTGGCCATCACATTCGTCAGAT-3'	-	1-25	e17, J04144
ACE/170RT	5'-CTTCCGTGGGACTCATGT-3'	T	23-40	i+5: 1-246
ACE/171RT	5'-TGCACCGTGAGGCTCTA-3'	T	136-152	i+8: 1-152
ACE/173F	5'-GCCCAATAGGAGGAAGCA-3'	MT	1-10, 1-9	i-2: 1-10, e2
ACE/174R	5'-CCCACCCCATCTCCAAGAA-3'	-	166-184	i-2: 1-184
ACE/175FB	5'-GCC-3'	MT, B	1-3	i-2: 1-10
ACE/176RT	5'-TCCCTGATGGGCTGCTCTC-3'	T	65-83	i-2: 1-184
ACE/177FT	5'-CAAGGCCCTCAACCAACTC-3'	T	1-19	i-24: 1-50
ACE/178RB	5'-TTCCCACAAAAGCTCCAGTG-3'	B	71-90	i+24: 1-108
ACE/179R	5'-GGCTCAAAATGGCAAGTGTT-3'	-	89-108	i+24: 1-108
ACE/180FT	5'-GGGCCATGTCCTTCTGACTC-3'	T	1-20	i-25: 1-45
ACE/181RB	5'-CAGCCTGGAGGGGTTAAGA-3'	B	33-51	i+25: 1-51
ACE/182R	5'-CCCTTCTGAGCGAGCTGAGT-3'	-	1-6,1-14	i-26: 1-6, e26, J04144
ACE/183F	5'-GGCCATGTTGAGCTACTTCAA-3'	-	83-103	e25, J04144
ACE/184FB	5'-CCTCCAGCCTTGGGTCTTAA-3'	B	19-38	i+25: 1-38
ACE/185RT	5'-TTCCCATCCCAGTCTCTGGT-3'	T	269-288	e26, J04144
ACE/188RT	5'-GGCAGCCTGGTTGATGAGT-3'	T	116-134	e17, J04144
ACE/192FB	5'-ATTCCAGCTCTGAAATTCTCTGA-3'	B	1-23	i-17: 1-85

Name	Sequence	Modification *)	Nucleotides	Numbering according to **)
ACP/3FT	5'-GAGCCCCTCCAGCACCTC-3'	T	499-5017	X94359
ACP/4RB	5'-ACCCGAGCCTGCCCACC-3'	B	5302-5318	X94359
ACP/5FT	5'-GGTCGGGCTGGGAAGATC-3'	T	5232-5249	X94359
ACP/6RB	5'-TCGGCTCTGCCCCTTCTC-3'	B	5576-5593	X94359 + additional downstream sequence
ACP/7FT	5'-GCCCTTTCTCCAGCTTCCTCT-3'	T	5361-5381	X94359
ACP/8RB	5'-CGGCGGCAGCAGCAACA-3'	B	5666-5682	X94359 + additional downstream sequence
ACP/11FB	5'-GAGCCCCTCCAGCACCTC-3'	B	499-5017	X94359
ACP/12RT	5'-ACCCGAGCCTGCCCACC-3'	T	5302-5318	X94359
ACP/13FB	5'-GGTCGGGCTGGGAAGATC-3'	B	5232-5249	X94359
ACP/14RT	5'-TCGGCTCTGCCCCTTCTC-3'	T	5576-5593	X94359 + additional downstream sequence
ACP/15FB	5'-GCCCTTTCTCCAGCTTCCTCT-3'	B	5361-5381	X94359
ACP/16RT	5'-CGGCGGCAGCAGCAACA-3'	T	5666-5682	X94359 + additional downstream sequence

Name	Sequence	Modification *)	Nucleotides	Numbering according to **)
ANG/1FT	5'-ATGGCACTTAAAGGTCAGTTAAT-3'	T	336-358	M24686
ANG/2RB	5'-TACGGAAGCCCAAGAAGTT-3'	B	726-745	M24686
ANG/5FT	5'-CTCCCCAACGGCTGTCTT-3'	T	797-814	M24686
ANG/6RB	5'-AGCAGCAACATCCAGTTCTGT-3'	B	1119-1139	M24686
ANG/7FT	5'-TCCCACGCTCTCTGGACTT-3'	T	1099-1117	M24686
ANG/8RB	5'-CTGATCTCAGCTACACATGGATACTA-3'	B	1290-1315	M24686
ANG/15FT	5'-CCTGTCTTGGGTGACTCTTC-3'	T	7-26	M24687
ANG/17FB	5'-TTCTGGGCTAAATGGTGACA-3'	B	285-304	M24686
ANG/18RT	5'-CTTGTCTTCGGTGTCAAGTTT-3'	T	675-695	M24686
ANG/19FB	5'-GGGAGCCTTGGACCACAC-3'	B	839-856	M24686
ANG/20RT	5'-AGCCTGCATGAACCTGTCAA-3'	T	1147-1167	M24686
ANG/21FB	5'-TGGTGGGCGTGTTCACA-3'	B	1018-1034	M24686
ANG/22RT	5'-GCCAGAGCCAGCAGAGA-3'	T	1264-1280	M24686
ANG/29RB	5'-CCACATTCCAGGGGAGAC-3'	B	335-352	M24687
ANG/30FB	5'-CCTGTCTTGGGTGACTCTTC-3'	B	7-26	M24687
ANG/32RT	5'-CCACATTCCAGGGGAGAC-3'	T	334-352	M24687
ANP/1FT	5'-GTCCCTTCAGTGCCCTAATAC-3'	T	314-334	X15232
ANP/2RB	5'-ACAGCCAGATTGAAAGACACA-3'	B	593-613	X15232
ANP/3FT	5'-AACCCTTTTACTGGTCATGTGA-3'	T	492-513	X15232
ANP/4RB	5'-CGCTCATGGGATGTGTGAC-3'	B	747-765	X15232
ANP/5FT	5'-TGTTTTCCCCAGTGTCTATTAGA-3'	T	686-708	X15232

Name	Sequence	Modification *)	Nucleotides	Numbering according to **)
ANP/6RB	5'-GCAGGGTCGAGTTACACATTT-3'	B	982-1003	X15232
ANP/7FT	5'-CCTCAGGCTGTCACACACCTA-3'	T	909-929	X15232
ANP/8RB	5'-CGGCTTACCTTCTGCTGTAGT-3'	B	1246-1266	X15232
ANP/9FB	5'-CTCCTTGAACCTGCTTGTGTT-3'	B	273-293	X15232
ANP/10RT	5'-GCATTGAAAGATGTGCTGTTCT-3'	T	548-569	X15232
ANP/11FB	5'-TAACGACTACAAAAGCAAGTCTTAC-3'	B	446-469	X15232
ANP/12RT	5'-AGAGGGCAGGGGAGAGTCT-3'	T	805-823	X15232
ANP/13FB	5'-GGCAGCAGGGTCAGAAGT-3'	B	766-783	X15232
ANP/14RT	5'-GCTGGAGAGGAGGGTTACAT-3'	T	1127-1146	X15232
ANP/15FB	5'-TGCAAACTTCGGTAAATGTGT-3'	B	970-990	X15232
ANP/16RT	5'-CAGAACAACGGCAGCTTCT-3'	T	1224-1242	X15232
AT1/5FT	5'-ACTGGCTGACTTATGCTTTTTACT-3'	T	547-570	S77410
AT1/6RB	5'-GGGTTGAATTTTGGGACTCATA-3'	B	884-905	S77410
AT1/7FT	5'-GCCAGTTTGCCAGCTATAAT-3'	T	809-828	S77410
AT1/8RB	5'-TGATGCCTAGTTGAATCAATACA-3'	B	1123-1145	S77410
AT1/9FT	5'-GAAGGCTTATGAAATTCAGAAGA-3'	T	1003-1025	S77410
AT1/10RB	5'-AAAGTCGGTTCAGTCCACATAA-3'	B	1535-1556	S77410
AT1/16FB	5'-AAACAGCTTGGTGGTGATAGTC-3'	B	469-490	S77410
AT1/17RT	5'-GCAGGTGACTTTGGCTACAA-3'	T	762-781	S77410
AT1/18FB	5'-CCTGTACGCTAGTGTGTTTCTACT-3'	B	667-690	S77410
AT1/19RT	5'-AGGAAACAGGAAACCCAGTATAT-3'	T	932-955	S77410

Name	Sequence	Modification *)	Nucleotides	Numbering according to **)
AT1/22FB	5'-CTGGATTCCCCACCAAATAT-3'	B	1090-1109	S77410
AT1/23RT	5'-TGCTCCTTCTTTCACAAAATTAC-3'	T	1438-1460	S77410
ATP/1FT	5'-CTTCCGTTATTATGTGTGATATTAGT-3'	T	1244-1269	U07144
ATP/2RB	5'-GCATGTACCTAAAAAGTCCTGTC-3'	B	1566-1588	U07144
ATP/5FT	5'-ATTGGCATATCCATCACCTTAA-3'	T	1628-1649	U07144
ATP/6RB	5'-GATCTCCCAACTCATGCTATGA-3'	B	1961-1982	U07144
ATP/7FT	5'-ATTGGATTCAATTTGCCTACAT-3'	T	1846-1867	U07144
ATP/8RB	5'-TTTGGTAATACAGTTGTGGATCATA-3'	B	2159-2184	U07144
ATP/9FT	5'-TGCAACTTGGGTAGCATGTC-3'	T	2077-2096	U07144
ATP/10RB	5'-AGTCGTCCCGTGTCAACTATC-3'	B	2370-2390	U07144
ATP/11FB	5'-CGTTGTCTTCCGTTATTATGTGT-3'	B	1238-1260	U07144
ATP/12RT	5'-TTATTGCATGTACCTAAAAAGTGTA-3'	T	1455-1479	U07144
ATP/15FB	5'-GCATTCATATAAAGATCAAATCAGT-3'	B	1600-1624	U07144
ATP/16RT	5'-CACCCTGATAACAAAACCAGATA-3'	T	1929-1951	U07144
ATP/17FB	5'-CTTTCTGGCATCAACCTCACT-3'	B	1794-1814	U07144
ATP/18RT	5'-ACTTTTAAGGACGAATTAGAGAACT-3'	T	2214-2238	U07144
ATP/19FB	5'-GTCCACCCTTGAATTTCATAAC-3'	B	2115-2136	U07144
ATP/20RT	5'-CCCAACCTCCTCCCTCTC-3'	T	2396-2413	U07144
ATP/21FT	5'-GCTCGCTCTCCCTCACGAC-3'	T	2310-2328	U07144
ATP/22RB	5'-TCCAGCCGCTCCCCATC-3'	B	2657-2673	U07144
ATP/23FB	5'-GCTCGCTCTCCCTCACGAC-3'	B	2310-2328	U07144

Name	Sequence	Modification *)	Nucleotides	Numbering according to **)
ATP/24RT	5'-TCCAGCCGCTCCCCATC-3'	T	2657-2673	U07144
ATR/1F	5'-GCCCCTCAGATAATGTAAGCTC-3'	-	1353-1374	S77410
ATR/2R	5'-AACCGGCACGAAAACTTTACT-3'	-	1834-1854	S77410
ATR/3aF	5'-GCACTTCACTACCAAATGAGCA-3'	-	1476-1500	S77410
ATR/4cF	5'-GCACTTCACTACCAAATGAGCC-3'	-	1476-1500	S77410

Where indicated, the primers were modified in one of the following ways: (i) a biotin molecule was conjugated to the 5' terminus of the indicated sequence (B); (ii) a sequence of nucleotides derived from M13, 5'-CAGGAAACAGCTATGACT-3', was added at the 5' terminus of the indicated sequence (MT); or (iii) the sequence 5'-AGTCACGACGTTGTAAAACGACGGCCAGT-3' was added at the 5' terminus of the indicated sequence (T = Tail). Nucleotides were numbered according to the Genbank sequences listed in Table 1 where indicated. When the sequences involved were not publicly available, the numbering was as in the following examples: The designation "i-4: 1-200" indicates that the primer sequence is located within the sequence extending 200 bp upstream of, and including, the nucleotide immediately upstream of the first coding nucleotide of exon 4. Similarly, the designation "i+4: 1-200" indicates that the primer sequence is located within the sequence extending from the nucleotide that is located immediately downstream of the last coding nucleotide of exon 4 downstream for 200 bp. In each case, the specific location of the primer sequence is indicated in Table 2 in the column marked "Nucleotides".

The reaction components used for PCR are described in Table 3 below.

Table 3

Condition	Components	Volume
A	Ultrapure dNTP Set 2.5 mM (dATP:dCTP:dGTP:dTTP = 1:1:1:1), (Pharmacia Biotech)	4 µl
	10xPCR buffer II, (Perkin Elmer)	5 µl
	$MgCl_2$ solution 2.5 mM,	3 µl
	AmpliTaq® DNApolymerase(Perkin Elmer) (5U/ml)	0.15 µl
	Primer 1	1 µl
	Primer 2	1 µl
	DNA solution	1 µl
	R/O-purified water q.s.	Tot. 50 µl
B	Ultrapure dNTP Set 2.5 mM (dATP:dCTP:dGTP:dITP:dTTP = 2:2:1:1:2), (Pharmacia Biotech)	4 µl
	10xPCR buffer II, (Perkin Elmer)	5 µl
	$MgCl_2$ solution 2.5 mM, (Perkin Elmer)	3 µl
	AmpliTaq® DNApolymerase (5U/ml)	0.15 µl
	Primer 1	1 µl
	Primer 2	1 µl
	DNA solution	1 µl
	R/O-purified water q.s.	Tot. 50 µl
C	Ultrapure dNTP Set 2.5 mM (dATP:dCTP:dGTP:dITP:dTTP = 4:4:1:3:4), (Pharmacia Biotech)	4 µl
	10xPCR buffer II, (Perkin Elmer)	5 µl

Condition	Components	Volume
	$MgCl_2$ solution 2.5 mM, (Perkin Elmer)	3 µl
	AmpliTaq® DNApolymerase (5U/ml)	0.15 µl
	Primer 1	1 µl
	Primer 2	1 µl
	DNA solution	1 µl
	R/O-purified water q.s.	Tot. 50 µl
D	Ultrapure dNTP Set 2.5 mM (dATP:dCTP:dGTP:dITP:dTTP = 6:6:1:5:6), (Pharmacia Biotech)	4 µl
	10xPCR buffer II, (Perkin Elmer)	5 µl
	$MgCl_2$ solution 2.5 mM, (Perkin Elmer)	3 µl
	AmpliTaq® DNApolymerase (5U/ml)	0.15 µl
	Primer 1	1 µl
	Primer 2	1 µl
	DNA solution	1 µl
	R/O-purified water q.s.	Tot. 50 µl
E	Ultrapure dNTP Set 2.5 mM (dATP:dCTP:dGTP:dITP:dTTP = 4:4:1:3:4), (Pharmacia Biotech)	4 µl
	10xPCR buffer II, (Perkin Elmer)	5 µl
	$MgCl_2$ solution 2.5 mM, (Perkin Elmer)	2.5 µl
	DMSO	2.5 µl
	AmpliTaqGold® DNApolymerase (5U/ml)	0.5 µl
	Primer 1	1 µl

Condition	Components	Volume
	Primer 2	1 µl
	DNA solution	1 µl
	R/O-purified water q.s.	Tot. 50 µl
F	Ultrapure dNTP Set 2.5 mM (dATP:dCTP:dGTP:dTTP = 1:1:1:1) (Pharmacia Biotech)	4 µl
	10xPCR buffer II, (Perkin Elmer)	5 µl
	$MgCl_2$ solution 2.5 mM, (Perkin Elmer)	2 µl
	AmpliTaq® DNApolymerase (5U/ml)	0.5 µl
	Primer 1	1 µl
	Primer 2	1 µl
	DNA solution	1 µl
	R/O-purified water q.s.	Tot. 50 µl
G	Ultrapure dNTP Set 2.5 mM (dATP:dCTP:dGTP:dTTP = 1:1:1:1) (Pharmacia Biotech)	4 µl
	10xPCR buffer II, (Perkin Elmer)	5 µl
	$MgCl_2$ solution 2.5 mM, (Perkin Elmer)	2 µl
	AmpliTaq® DNApolymerase (5U/ml)	0.5 µl
	Primer 1	1 µl
	Primer 2	1 µl
	DNA solution	1 µl

Condition	Components	Volume
	R/O-purified water q.s.	Tot. 50 µl
H	Ultrapure dNTP Set 2.5 mM (dATP:dCTP:dGTP:dITP:dTTP = 4:4:1:3:4), (Pharmacia Biotech)	4 µl
	10xPCR buffer II, (Perkin Elmer)	5 µl
	$MgCl_2$ solution 2.5 mM, (Perkin Elmer)	4 µl
	AmpliTaqGold® DNApolymerase (5U/ml)	0.5 µl
	Primer 1	1 µl
	Primer 2	1 µl
	DNA solution	1 µl
	R/O-purified water q.s.	Tot. 50 µl

The reaction conditions used for PCR are described in Table 4 below.

Table 4

PCR-method	Temperature*)	Time *)	Temperature **)	Time	Temperature	Time	No of cycles ***)
25	94	15 s	55	30 s	72	45 s	35
	72	5 min					1
	22	∞					
27	94	15 s	55	30 s	72	45 s	35
	72	5 min					1
	22	∞					
36	94	2 min					1
	94	15 s	58	30 s	72	45 s	35
	72	5 min					1
	22	∞					
38	94	2 min					1
	94	15 s	60	30 s	72	45 s	15
	72	5 min					1
	22	∞					
40	94	2 min					1

PCR-method	Temperature*)	Time *)	Temperature **)	Time	Temperature	Time	No of cycles ***)
	94	15 s	60	30 s	72	45 s	35
	72	5 min					1
	22	∞					
54	96	5 min					1
	96	30 s	61	30 s	72	45 s	15
	72	5 min					1
	22	∞					
56	96	5 min					1
	96	30 s	61	30 s	72	45 s	35
	72	5 min					1
	22	∞					
64	95	2 min					
	95	15 s	59	30 s	72	45 s	40
	72	5 min					1
	22	∞					
70	95	5 min					
	95	15 s	59	30 s	72	45 s	50
	72	5 min					1

PCR-method	Temperature*)	Time *)	Temperature **)	Time	Temperature	Time	No of cycles ***)
	22	∞					

All temperatures are given in degrees Celsius.

*) indicates the default initial temperatures (°C) and times of the program.
**) indicates the default temperature (°C) of the program.
***) indicates the default number of cycles of the program, referring to the section of the PCR program where three different temperatures are employed.

Any differences are indicated in "Modifications" in Table 5 below.

The amplified fragments are described in Table 5 below with respect to the primers and PCR reaction conditions used for amplification.

Table 5

Fragment	Primer 1	Primer 2	PCR method	Modifications of PCR method	PCR reaction conditions
ANPf1F	ANP/1FT	ANP/2RB	64		A
ANPf2F	ANP/3FT	ANP/4RB	64		B
ANPf3F	ANP/5FT	ANP/6RB	64	anneal. temp: 48°C	A
ANPf4F	ANP/7FT	ANP/8RB	64	anneal. temp: 59°C	D
ANPf5R	ANP/9FB	ANP/10RT	64		A
ANPf6R	ANP/11FB	ANP/12RT	64		B

Fragment	Primer 1	Primer 2	PCR method	Modifications of PCR method	PCR reaction conditions
ANPf7R	ANP/13FB	ANP/14RT	64		A
ANPf8R	ANP/15FB	ANP/16RT	64		C
ANGe2f1F	ANG/1FT	ANG/2RB	64		C
ANGe2f3F	ANG/5FT	ANG/6RB	64		C
ANGe2f4F	ANG/7FT	ANG/8RB	64		A
ANGe2f5R	ANG/17FB	ANG/18RT	64		A
ANGe2f7R	ANG/19FB	ANG/20RT	64		A
ANGe2f8R	ANG/21FB	ANG/22RT	64		A
ANGe3F	ANG/15FT	ANG/29RB	64	anneal. temp: 57°C	F
ANGe3R	ANG/30FB	ANG/32RT	64	anneal. temp: 57°C, 45 cycles	A
ACPf2F	ACP/3FT	ACP/4RB	70	anneal. temp: 62°C	E
ACPf3F	ACP/5FT	ACP/6RB	70	anneal. temp: 58°C	E
ACPf4F	ACP/7FT	ACP/8RB	70		E
ACPf6R	ACP/11FB	ACP/12RT	70	anneal. temp: 62°C	E
ACPf7R	ACP/13FB	ACP/14RT	70	anneal. temp: 58°C	E
ACPf8R	ACP/15FB	ACP/16RT	70		E
ACEe2R PCR1	ACE/173F	ACE/174R	38		A
ACEe2R PCR2	ACE/175FB	ACE/176RT	40		A
ACEe4F PCR1	ACE/119FB	ACE/120RB	27		A
ACEe4F PCR2	ACE/119FT	ACE/123RB	25		A
ACEe5R PCR1	ACE/119FB	ACE/120RB	27		A
ACEe5R PCR2	ACE/122FB	ACE/170RT	25		A

Fragment	Primer 1	Primer 2	PCR method	Modifications of PCR method	PCR reaction conditions
ACEe7F PCR1	ACE/145F	ACE/114RB	27		A
ACEe7F PCR2	ACE/118FT	ACE/114RB	25		A
ACEe8R PCR1	ACE/130F	ACE/134RB	27		A
ACEe8R PCR2	ACE/130FB	ACE/171RT	25		A
ACEe15R PCR1	ACE/107F	ACE/108RB	27		A
ACEe15R PCR2	ACE/107FB	ACE/111RT	25		A
ACEe17R	ACE/192FB	ACE/188RT	40	anneal. temp: 63°C, 40 cycles	A
ACEe19F PCR1	ACE/84FT	ACE/79RB	27		A
ACEe19F PCR2	ACE/84FT	ACE/82RB	25		A
ACEe21R PCR1	ACE/94FB	ACE/95RB	27		A
ACEe21R PCR2	ACE/94FB	ACE/96RT	25		A
ACEe24F PCR1	ACE/177FT	ACE/179R	38		A
ACEe24F PCR2	ACE/177FT	ACE/178RB	40		A
ACEe25F PCR1	ACE/180FT	ACE/182R	38		A
ACEe25F PCR2	ACE/180FT	ACE/181RB	40		A

Fragment	Primer 1	Primer 2	PCR method	Modifications of PCR method	PCR reaction conditions
ACEe26R PCR1	ACE/183F	ACE/185RT	54		A
ACEe26R PCR2	ACE/184FB	ACE/185RT	56		A
ACEDI	ACE/146F	ACE/147R	36		A
ATPf1F	ATP/1FT	ATP/2RB	64		A
ATPf3F	ATP/5FT	ATP/6RB	64	anneal. temp: 58°C	A
ATPf4F	ATP/7FT	ATP/8RB	64	anneal. temp: 48°C	A
ATPf5F	ATP/9FT	ATP/10RB	64	anneal. temp: 58°C	A
ATPf6R	ATP/11FB	ATP/12RT	64	anneal. temp: 48°C	A
ATPf8R	ATP/15FB	ATP/16RT	64	anneal. temp: 55°C	G
ATPf9R	ATP/17FB	ATP/18RT	64	anneal. temp: 54°C	A
ATPf10R	ATP/19FB	ATP/20RT	64		A
ATPf11F	ATP/21FT	ATP/22RB	64	initial denaturation: 95°C, 12 min.	H
ATPf12R	ATP/23FB	ATP/24RT	64	initial denaturation: 95°C, 12 min.	H
AT1e5f2F	AT1/5FT	AT1/6RB	64		A
AT1e5f3F	AT1/7FT	AT1/8RB	64		A
AT1e5f4F	AT1/9FT	AT1/10RB	64		C
AT1e5f6R	AT1/16FB	AT1/17RT	64		A
AT1e5f7R	AT1/18FB	AT1/19RT	64		C
AT1e5f9R	AT1/22FB	AT1/23RT	64		A

Fragment	Primer 1	Primer 2	PCR method	Modifications of PCR method	PCR reaction conditions
AT1-spec. 1	ATR/1F ATR/3aF	ATR/2R	40	anneal. temp: 63°C	A
AT1-spec. 2	ATR/1F ATR/2R	ATR/4cF	40	anneal. temp: 63°C	A

All of the PCR products (except fragments ACEDI, AT1-spec. 1 and AT1-spec. 2) were subjected to solid phase sequencing according to the protocol commercially available from Pharmacia Biotech. The sequencing reactions are performed with a sequencing primer having a complementary sequence to the "Tail" sequence previously described in Table 2. The nucleotide sequence of the sequencing primer was 5'-CGACGTTGTAAAACGACGGCCAGT-3', and the primer was fluorescently labeled with a Cy-5-molecule on the 5'-nucleotide. The positions carrying a genetic variation were identified by determination of the nucleotide sequence by the use of the ALFexpress™ system commercially available from Pharmacia Biotech.

The detection of the fragment ACEDI was performed by analyzing the sizes of the amplified fragments by gel electrophoresis, where the presence of a shorter PCR product (192 base pairs) indicated the D-allele and a longer PCR product (479 base pairs) indicated the I-allele. The presence of both bands indicated a heterozygote for the two alleles. The detection of the allele-specific reaction of position AT1-1271 was performed by separately running two parallel PCR reactions on the same sample and comparing the sizes of the amplified fragments. A PCR product of 501 base pairs should always be present as a control in both parallel runs, whereas the presence of a PCR product of 378 base pairs in the reaction designated AT1-spec. 1 indicated the presence of an A in this position. The presence of a PCR product of 378 base pairs in the reaction designated AT1-spec. 2 indicated a C in this position. If the shorter PCR product was present in both reactions, the individual is a heterozygote for A and C.

Results:

The analysis described above resulted in the identification of polymorphic positions within the regulatory and coding/intron segments of the human genes encoding ACE, AGT, and AT1. The polymorphic positions, the variant nucleotides found at each of the positions, and the PCR fragment in which the polymorphism was identified are shown in Table 6 below. Also shown are the frequencies of each genotype in a population of 90 individuals, expressed as the percent of the study population having that genotype. Polymorphisms that resulted in alternate amino acids in ACE, AGT, or AT1 are also indicated. As used herein below, the designations "AGR" , "ACR", and "ATR" refer to the

regulatory regions of the human AGT, ACE, and AT1 genes, respectively; and the designations "AGT", "ACE", and "AT1", refer to the coding regions of the AGT, ACE, and AT1 genes.

Table 6

Gene	Position	Reported genotype	Genetic variation	Frequency (per cent)	Amino acid change	Fragment	Reference (if any)
AGR	395	T	TT-TA-AA	88-11-1	None	ANPf1F ANPf5R	-
AGR	412	C	CC-CT	99-1	None	ANPf1F ANPf5R	-
AGR	432	G	GG-GA	81-19	None	ANPf1F ANPf5R	-
AGR	449	C	TT-TC	92-8	None	ANPf1F ANPf5R	-
AGR	692	C	CC-CT	81-19	None	ANPf2F ANPf6R	-
AGR	839	G	GG-GA	93-7	None	ANPf3F ANPf7R	-
AGR	1007	G	GG-GA	81-19	None	ANPf4F ANPf7R	-
AGR	1072	G	GG-GA	89-11	None	ANPf4F ANPf7R	-
AGR	1204	C	CC-CA-AA	67-33	None	ANPf4F ANPf8R	-
AGR	1218	A	AA-AG-GG	14-55-31	None	ANPf4F ANPf8R	Inuoe, I et. al. J. C. I. (1997) 99: 1786-1789.
AGT	273	C	CC-CT	99-1	None	ANGe2f1F ANGe2f5R	-

Gene	Position	Reported genotype	Genetic variation	Frequency (per cent)	Amino acid change	Fragment	Reference (if any)
AGT	620	C	CC-CT	80-20	Thr - Met	ANGe2f3F ANGe2f7R	JeunmaîtreX, et al. Cell (1992) 71:169-180.
AGT	803	T	TT-TC-CC	35-52-13	Met - Thr	ANGe2f4F ANGe2f8R	JeunmaîtreX, et al. Cell (1992) 71:169-180.
AGT	912	C	CC-CT	99-1	None	ANGe3F ANGe3R	-
AGT	997	G	CC	100	Glu - Gln	ANGe3F ANGe3R	-
AGT	1116	G	GG-GA-AA	87-12-1	None	ANGe3F ANGe3R	-
AGT i3	49 Numbering according to GenBank entry M24688	A	AA-AG	80-20	None	ANGe3F ANGe3R	-
AGT	1174	C	CC-CA	99-1	Leu - Met	ANGe4F ANGe4R	-
ACR	5106	C	CC-CT	98-2	None	ACPf2F ACPf6R	-
ACR	5349	A	AA-AT-TT	35-46-19	None	ACPf3F ACPf7R	Villard, E. et al. Am. J. Hum. Genet. (1996) 58: 1268-1278
ACR	5496	T	TT-TC-CC	35-46-19	None	ACPf4F ACPf8R	Villard, E. et al. Am. J. Hum. Genet. (1996) 58: 1268-1278

Gene	Position	Reported genotype	Genetic variation	Frequency (per cent)	Amino acid change	Fragment	Reference (if any)
ACE	375	A	CC	100	None	ACEe2R	-
ACE	582	C	CC-CT	94-6	None	ACEe4F	-
ACE	731	A	AA-AG	96-4	Tyr - Cys	ACEe5R	-
ACE	1060	G	GG-GA	97-3	Gly - Arg	ACEe7F	-
ACE	1215	C	CC-CT-TT	35-42-23	None	ACEe8R	Villard, E. et al. Am. J. Hum. Genet. (1996) 58: 1268-1278
ACE	2193	G	GG-GA-AA	20-57-23	None	ACEe15R	Villard, E. et al. Am. J. Hum. Genet. (1996) 58: 1268-1278
ACE	1451		DD-DI-II	29-54-20	None	ACEDI	Villard, E. et al. Am. J. Hum. Genet. (1996) 58: 1268-1278
ACE	2328	A	AA-AG-GG	20-57-23	None	ACEe17R	Villard, E. et al. Am. J. Hum. Genet. (1996) 58: 1268-1278
ACE	2741	G	TT	100	Gly - Val	ACEe19F	-
ACE	3132	C	CC-CT	99-1	None	ACEe21R	-
ACE	3387	T	TT-TC-CC	20-57-23	None	ACEe24F	-
ACE	3503	C	GG	100	Ala - Gly	ACEe25F	-
ACE	3906	G	GG-GA	91-9	None	ACEe26R	-
ATR	1427	A	AA-AT-TT	77-22-1	None	ANPf1F ANPf6R	-
ATR	1756	T	TT-TA-AA	75-24-1	None	ANPf3F ANPf8R	-

Gene	Position	Reported genotype	Genetic variation	Frequency (per cent)	Amino acid change	Fragment	Reference (if any)
ATR	1853	T	TT-TG-GG	82-11-1	None	ANPf3F ANPf8R	-
ATR	2046	T	CC-CT-TT	46-46-8	None	ANPf4F ANPf9R	-
ATR	2354	A	AA-AC-CC	73-26-1	None	ANPf5F ANPf10R	-
ATR	2355	G	GG-GC-CC	73-26-1	None	ANPf5F ANPf10R	-
ATR	2415	A	AA-AG	75-24-1	None	ANPf11F ANPf12R	-
AT1	449	G	GG-GC	99-1	Ser - Thr	AT1e5f2F AT1e5f6R	-
AT1	678	T	CC-CT-TT	31-48-21	None	AT1e5f3F AT1e5f7R	Rolfs A, et. al. Eur. Heart. J. (1994) 15: Suppl. D, 108-112.
AT1	1167	A	AA-AG	92-8	None	AT1e5f4F AT1e5f9R	Rolfs A, et. al. Eur. Heart. J. (1994) 15: Suppl. D, 108-112.
AT1	1271	A	AA-AC-CC	50-40-10	None	AT1-spec	Bonnardeaux, A. et al. Hypertension (1994) 24:63-69.

A subset of these polymorphic positions were further analyzed in an additional 187 individuals. Table 7 shows the polymorphic positions, the sequence at these positions, and the genotype frequencies for each position in a population of 277 as described in Example 1 above.

Table 7

Gene	Position	Genetic variation	Frequency (per cent)
AGR	395	TT-TA-AA	87-12-7
AGR	432	GG-GA-AA	78-21-1
AGR	449	TT-TC-CC	94-5-1
AGR	692	CC-CT-TT	78-21-1
AGR	839	GG-GA	96-4
AGR	1007	GG-GA-AA	78-21-1
AGR	1072	GG-GA	76-24
AGR	1204	CC-CA-AA	3-27-70
AGR	1218	AA-AG-GG	16-50-34
AGT	620	CC-CT-TT	75-23-2
AGT	803	TT-TC-CC	34-50-16
AGT	1116	GG-GA-AA	83-15-2
ACR	5349	AA-AT-TT	37-44-19
ACR	5496	TT-TC-CC	38-43-19
ACE	1060	GG-GA	96-4
ACE	1215	CC-CT-TT	34-46-20
ACE	2193	GG-GA-AA	22-53-25
ACE	2328	AA-AG-GG	23-52-25
ACE	3387	TT-TC-CC	24-53-23
ACE	3906	GG-GA-AA	86-13-1
ATR	1427	AA-AT-TT	72-26-2
ATR	1756	TT-TA-AA	72-25-3
ATR	1853	TT-TG-GG	73-25-2
ATR	2046	CC-CT-TT	47-41-12
ATR	2354	AA-AC-CC	72-26-2
ATR	2355	GG-GC-CC	71-27-2

Gene	Position	Genetic variation	Frequency (per cent)
ATR	2415	AA-AG-GG	73-25-2
AT1	678	CC-CT-TT	26-51-23
AT1	1167	AA-AG	88-12
AT1	1271	AA-AC-CC	55-36-9

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Example 2: Correlation of Polymorphic Patterns with Cardiovascular Disease

The polymorphic positions identified as in Example 1 were correlated with the following markers of cardiovascular status present in the study population: myocardial infarction (MI); stroke; and high blood pressure. Polymorphic patterns, i.e., combinations of sequences at particular polymorphic positions, that show a statistically significant correlation with one or more of these markers are shown below.

ACR 5349 A/T, AGR 1218 A

	Healthy (100)	MI (120)	Stroke (37)	High BP (39)	Total (n)
# of events	3	7	3	5	17
% within group	3	5.8	8.1	12.8	

ACR 5496 C, AGR 1204 A/C

	Healthy (100)	MI (120)	Stroke (37)	High BP (39)	Total (n)
# of events	2	7	3	2	13
% within group	2	5.8	8.1	5.1	

ACR 5496 C/T, AGR 1218 A, AGT 620 C/T

	Healthy (100)	MI (120)	Stroke (37)	High BP (39)	Total (n)
# of events	4	13	1	3	21
% within group	4	10.8	2.7	7.7	

ACE 2193 A, AGR 1204 C, ACE 2328 G

	Healthy (100)	MI (120)	Stroke (37)	High BP (39)	Total (n)
# of events	0	11	3	3	16
% within group	0	9.2	8.1	7.7	

ACE 2193 A, AGR 1204 A/C

	Healthy (100)	MI (120)	Stroke (37)	High BP (39)	Total (n)
# of events	1	1	0	1	3
% within group	1	0.8	0	2.6	

ACE 3387 T, AGR 1218 A

	Healthy (100)	MI (120)	Stroke (37)	High BP (39)	Total (n)
# of events	2	4	1	3	10
% within group	2	3.3	2.7	7.7	

ACE 3387 T, AGT 620 C/T

	Healthy (100)	MI (120)	Stroke (37)	High BP (39)	Total (n)
# of events	1	10	3	2	15
% within group	1	8.3	8.1	5.1	

AGR 1204 A/C, AT1 678 C/T

	Healthy (100)	MI (120)	Stroke (37)	High BP (39)	Total (n)
# of events	5	23	5	6	37
% within group	5	19.2	13.5	15.4	

AGR 1204 A/C, AT1 1271 A/C

	Healthy (100)	MI (120)	Stroke (37)	High BP (39)	Total (n)
# of events	3	17	3	4	26
% within group	3	14.2	8.1	10.3	

ACE 1215 C, AGR 1204 A/C

	Healthy (100)	MI (120)	Stroke (37)	High BP (39)	Total (n)
# of events	3	13	5	6	25
% within group	3	10.8	13.5	15.4	

AGR 1204 A/C, AT1 1167 A, ACE 3906 A/G

	Healthy (100)	MI (120)	Stroke (37)	High BP (39)	Total (n)
# of events	0	5	1	0	6
% within group	0	4.2	2.7	0	

AGR 1204 A, AGT 620 C, AT1 1271 A, AT1 1167 A, AGR 395 A/T

	Healthy (100)	MI (120)	Stroke (37)	High BP (39)	Total (n)
# of events	1	4	5	3	11
% within group	1	3.3	13.5	7.7	

AGR 1204 A/C, AGT 620 C/T, AT1 1271 A/C, AT1 1167 A, AGR 395 T

	Healthy (100)	MI (120)	Stroke (37)	High BP (39)	Total (n)
# of events	3	13	3	2	20
% within group	3	10.8	8.1	5.1	

AGR 1204 A/C, AGT 620 C/T, AT1 1271 A/C, AT1 1167 A/G, AGR 395 T

	Healthy (100)	MI (120)	Stroke (37)	High BP (39)	Total (n)
# of events	0	2	0	1	3
% within group	0	1.7	0	2.6	

Summary of the three previous polymorphic patterns (which involve the same polymorphic positions):

	Healthy (100)	MI (120)	Stroke (37)	High BP (39)	Total (n)
# of events	4	19	8	6	34
% within group	4	15.8	21.6	15.4	

AGR 1204 A, AT1 678 C, AT1 1167 A, AGR 395 A/T

	Healthy (100)	MI (120)	Stroke (37)	High BP (39)	Total (n)
# of events	1	2	2	1	5
% within group	1	1.7	5.4	2.6	

AGR 1204 A/C, AT1 678 C/T, AT1 1167 A, AGR 395 T

	Healthy (100)	MI (120)	Stroke (37)	High BP (39)	Total (n)
# of events	3	18	5	4	28
% within group	3	15.0	13.5	10.3	

Summary of the two previous polymorphic patterns:

	Healthy (100)	MI (120)	Stroke (37)	High BP (39)	Total (n)
# of events	4	20	7	5	33
% within group	4	16.7	18.9	12.8	

AGT 620 C/T, AT1 1271 A/C, AT1 1167 A, AGR 395 T

	Healthy (100)	MI (120)	Stroke (37)	High BP (39)	Total (n)
# of events	2	8	1	2	13
% within group	2	6.7	2.7	5.1	

AGT 620 C/T, AT1 1271 A/C, AT1 1167 A/G, AGR 395 T

	Healthy (100)	MI (120)	Stroke (37)	High BP (39)	Total (n)
# of events	0	2	0	1	3
% within group	0	1.7	0	2.6	

AGT 620 C, AT1 1271 A, AT1 1167 A, AGR 395 A/T

	Healthy (100)	MI (120)	Stroke (37)	High BP (39)	Total (n)
# of events	1	4	5	3	11
% within group	1	3.3	13.5	7.7	

Summary of the three previous polymorphic patterns:

	Healthy (100)	MI (120)	Stroke (37)	High BP (39)	Total (n)
# of events	3	14	6	6	27
% within group	3	11.7	16.2	15.4	

AGT 620 C, AT1 678 A, AT1 1167 A, AGR 395 A/T

	Healthy (100)	MI (120)	Stroke (37)	High BP (39)	Total (n)
# of events	1	2	2	1	5
% within group	1	1.7	5.4	2.6	

AGT 620 C/T, AT1 678 C/T; AT1 1167 A, AGR 395 T

	Healthy (100)	MI (120)	Stroke (37)	High BP (39)	Total (n)
# of events	3	15	4	4	24
% within group	3	12.5	10.8	10.3	

Summary of the two previous polymorphic patterns:

	Healthy (100)	MI (120)	Stroke (37)	High BP (39)	Total (n)
# of events	4	17	6	5	29
% within group	4	14.2	16.2	12.9	

ACE 2193 A, AGR 1218 A, AGT 803 A

	Healthy (100)	MI (120)	Stroke (37)	High BP (39)	Total (n)
# of events	2	5	1	3	11
% within group	2	4.2	2.7	7.7	

ACE 2193 A, AGT 620 C/T

	Healthy (100)	MI (120)	Stroke (37)	High BP (39)	Total (n)
# of events	1	11	3	2	16
% within group	1	9.2	8.1	5.1	

ACE 2328 G, AGT 620 C/T

	Healthy (100)	MI (120)	Stroke (37)	High BP (39)	Total (n)
# of events	1	11	3	2	16
% within group	1	9.2	8.1	5.1	

ACE 3387 T, AGR 1204 A/C

	Healthy (100)	MI (120)	Stroke (37)	High BP (39)	Total (n)
# of events	0	10	3	3	15
% within group	0	8.3	8.1	7.7	

Example 3: Correlation Between a Specific Polymorphic Pattern and Treatment Response

The following study was undertaken to define polymorphic patterns in the human ACE, AGT, and/or AT1 genes that predict the efficacy of treatments for cardiovascular disease.

Two groups of hypertensive patients were studied, 41 in the first group and 20 in the second group. The groups were analyzed independently and in combination.

The patients in this population were each treated with one of the following five ACE inhibitors: Captopril, Trandolapril, Lisinopril, Fosinopril, or Enalapril. The effect of the drugs on mean arterial blood pressure was quantified. Mean arterial blood pressure was defined as 2/3 of the diastolic blood pressure + 1/3 of systolic blood pressure. The individuals were also categorized as "high responders," i.e., those exhibiting a decrease of more than 16 mm Hg during treatment with an ACE inhibitor drug, and "low responders," i.e., those not exhibiting a decrease of more than 16 mm Hg.

One particular polymorphic pattern, ACE 2193 A/G, AGR 1072 G/G, AT1 1167 A/A, which was present in 51% of the first study population, discriminated between high responders and low responders. In the second group of 20 patients, the pattern was less prevalent (25%), but the correlation with lowered blood pressure was evident. Individuals having this polymorphic pattern (designated "1" below) experienced a larger decrease in blood pressure than those lacking this polymorphic pattern (designated "0" below).

Polymorphic Pattern	Observations	Mean (mm Hg) Change in B.P.	S.D.
0	36	-11.4	8.6
1	25	-18.1	9.7

Furthermore, the distribution of high responders and low responders (as defined above) was as follows:

Polymorphic Pattern	Low responder %	High responder %
0	80.1	19.4
1	24.0	76.0

Taken together, the results from the two groups indicate that the presence of this polymorphic pattern correlates with an incremental decrease of 6.4-7.3 mm Hg relative to individuals not having this polymorphic pattern.

The prevalence of this polymorphic pattern was 41% in this hypertensive population. This suggests that testing for this polymorphic pattern in hypertensive patients, followed by prescribing ACE inhibitors only to those patients having this polymorphic pattern, could increase the response rate from 43% (in a hypertensive population in general) to 76% in hypertensive population selected according to the methods of the invention.

Claims:

1. A method for assessing cardiovascular status in a human individual, said method comprising

(i) determining the sequence of one or more polymorphic positions within one or more of the ACE, AGT, or AT1 genes of said individual to establish a polymorphic pattern for said individual; and

(ii) comparing the polymorphic pattern established in (i) with the polymorphic patterns of humans exhibiting predetermined markers of cardiovascular status.

2. A method as defined in claim 1, wherein said predetermined status markers are selected from the group consisting of: blood pressure, electrocardiographic profile, and diagnosis of a cardiovascular syndrome.

3. A method as defined in claim 2, wherein said syndrome is selected from the group consisting of myocardial infarction, hypertension, atherosclerosis, and stroke.

4. A method as defined in claim 2, wherein said predetermined status markers indicate response to a cardiovascular treatment regimen.

5. A method as defined in claim 4, wherein said treatment regimen comprises administering a cardiovascular drug selected from the group consisting of ACE inhibitors, angiotensin II receptor antagonists, diuretics, alpha-adrenoreceptor antagonists, cardiac glycosides, phosphodiesterase inhibitors, beta-adrenoreceptor antagonists, calcium channel blockers, HMG-CoA reductase inhibitors, imidizoline receptor blockers, endothelin receptor blockers, and organic nitrites.

6. A method as defined in claim 5, wherein said one or more polymorphic positions comprise ACE 2193, AGR 1072, and AT1 1167.

7. A method as defined in claim 6, wherein said polymorphic pattern comprises ACE 2193 A/G, AGR 1072 G/G, and AT1 1167 A/A.

8. A method for predicting response of an individual suffering from a cardiovascular syndrome to treatment with an ACE inhibitor, said method comprising

(i) determining the sequence of (a) the ACE gene at position 2193 in the coding region; (b) the AGT gene at position 1072 in the regulatory region; and (c) the AT1 gene at position 1167 in the coding region, to establish a polymorphic pattern for said individual comprising said positions; and

(ii) comparing the polymorphic pattern established in (i) with the polymorphic patterns of humans exhibiting different responses to said ACE inhibitor.

9. A method as defined in claim 8, wherein said polymorphic pattern comprises ACE 2193 A/G, AGR 1072 G/G, AT1 1167 A/A.

10. A method as defined in claim 1, wherein said polymorphic position is selected from the group consisting of positions in the ACE regulatory region numbered 5106, 5349, and 5496; positions in the ACE coding region numbered 375, 582, 731, 1060, 1215, 2193, 2328, 2741, 3132, 3387, 3503, and 3906; position 1451 in the ACE gene as numbered in Genbank entry X62855; positions in the AGT regulatory region numbered 395, 412, 432, 449, 692, 839, 1007, 1072, 1204, and 1218; positions in the AGT coding region numbered 273, 620, 803, 912, 997, 1116, and 1174; position 49 in the AGT gene as numbered in Genbank entry M24688; positions in the AT1 regulatory region numbered 1427, 1756, 1853, 2046, 2354, 2355, and 2415; positions in the AT1 coding region numbered 449, 678, 1167, and 1271; and combinations of any of the foregoing.

11. A method as defined in claim 10, wherein said polymorphic patterns are selected from the group consisting of: ACR 5349 A/T, AGR 1218 A; ACR 5496 C, AGR 1204 A/C; ACR 5496 C/T, AGR 1218 A, AGT 620 C/T; ACE 2193 A, AGR 1204

C, ACE 2328 G; ACE 2193 A, AGR 1204 A/C; ACE 3387 T, AGR 1218 A; ACE 3387 T, AGT 620 C/T; AGR 1204 A/C, AT1 678 C/T; AGR 1204 A/C, AT1 1271 A/C; ACE 1215 C, AGR 1204 A/C; AGR 1204 A/C, AT1 1167 A, ACE 3906 A/G; AGR 1204 A, AGT 620 C, AT1 1271 A, AT1 1167 A, AGR 395 A/T; AGR 1204 A/C, AGT 620 C/T, AT1 1271 A/C, AT1 1167 A, AGR 395 T; AGR 1204 A/C, AGT 620 C/T, AT1 1271 A/C, AT1 1167 A/G, AGR 395 T; AGR 1204 A, AT1 678 C, AT1 1167 A, AGR 395 A/T; AGR 1204 A/C, AT1 678 C/T, AT1 1167 A, AGR 395 T; AGT 620 C/T, AT1 1271 A/C, AT1 1167 A, AGR 395 T; AGT 620 C/T, AT1 1271 A/C, AT1 1167 A/G, AGR 395 T; AGT 620 C, AT1 1271 A, AT1 1167 A, AGR 395 A/T; AGT 620 C, AT1 678 A, AT1 1167 A, AGR 395 A/T; AGT 620 C/T, AT1 678 C/T; AT1 1167 A, AGR 395 T; ACE 2193 A, AGR 1218 A, AGT 803 A; ACE 2193 A, AGT 620 C/T; ACE 2328 G, AGT 620 C/T; ACE 3387 T, AGR 1204 A/C; ACE 2193 A, ACE 2328 G, AGR 1204 C; and ACE 2193 A/G, AGR 1072 G/G, AT1 1167 A/A.

12. An isolated nucleic acid encoding ACE in an individual, wherein said nucleic acid comprises a polymorphic position and wherein the presence of said polymorphic position, either alone or in combination with other polymorphic positions in the sequence of human ACE or in one or more other human genes, predicts the cardiovascular status of said individual.

13. An isolated nucleic acid encoding AGT in an individual, wherein said nucleic acid comprises a polymorphic position and wherein the presence of said polymorphic position, either alone or in combination with other polymorphic positions in the sequence of human AGT or in one or more other human genes, predicts the cardiovascular status of said individual.

14. An isolated nucleic acid encoding AT1 in an individual, wherein said nucleic acid comprises a polymorphic position and wherein the presence of said polymorphic position, either alone or in combination with other polymorphic positions in

the sequence of human AT1 or in one or more other human genes, predicts the cardiovascular status of said individual.

15. An isolated nucleic acid derived from the human gene encoding angiotensin coverting enzyme (ACE), wherein said nucleic acid comprises a polymorphic position selected from the group consisting of A position in the regulatory region numbered 5106; positions in the coding region numbered 375, 582, 731, 1060, 2741, 3132, 3387, 3503, and 3906; position 1451 as numbered in Genbank entry X62855; and combinations of any of the foregoing.

16. A nucleic acid as defined in claim 15 wherein the sequence at said polymorphic position in the regulatory region is selected from the group consisting of 5106C and 5106T; and the sequence at said polymorphic position in the coding region is selected from the group consisting of 375A, 375C, 582C, 582T, 731A, 731G, 1060G, 1060A, 2741G, 2741T, 3132C, 3132T, 3387T, 3387C, 3503G, 3503C, 3906G, 3906A, and a deletion of nucleotides 1451-1783 as numbered in Genbank entry X62855.

17. A probe which hybridizes at high stringency to a polymorphic position as defined in claim 15.

18. An isolated nucleic acid comprising the human gene encoding angiotensinogen (AGT), wherein said nucleic acid comprises a polymorphic position selected from the group consisting of positions in the regulatory region numbered 395, 412, 432, 449, 692, 839, 1007, 1072, and 1204; positions in the coding region numbered 273, 912, 997, 1116, 1174, and position 49 in Genbank entry M24688; and combinations of any of the foregoing.

19. A nucleic acid as defined in claim 18 wherein the sequence at said polymorphic position in the regulatory region is selected from the group consisting of 395T, 395A, 412C, 412T, 432G, 432A, 449T, 449C, 692C, 692T, 839G, 839A, 1007G,

1007A, 1072G, 1072A, 1204C, and 1204A; and the sequence at the polymorphic position in the coding region is selected from the group consisting of 273C, 273T, 912C, 912T, 997G, 997C, 1116G, 1116A, 1174C, and 1174A, and A or G at position 49 in Genbank entry M24688.

20. A probe which hybridizes at high stringency to a polymorphic position as defined in claim 18.

21. An isolated nucleic acid comprising the human gene encoding type I angiotensin II receptor (AT1), wherein said nucleic acid comprises a polymorphic position selected from the group consisting of positions in the regulatory region numbered 1427, 1756, 1853, 2046, 2354, 2355, and 2415; a position in the coding/intron region numbered 449; and combinations of the foregoing.

22. A nucleic acid as defined in claim 21 wherein the sequence at said polymorphic position in the regulatory region is selected from the group consisting of 1427A, 1427T, 1756T, 1756A, 1853T, 1853G, 2046T, 2046C, 2354A, 2354C, 2355G, 2355C, 2415A and 2415G; and the sequence at said polymorphic position in the coding/intron region is selected from the group consisting of 449G and 449C.

23. A probe which hybridizes at high stringency to a polymorphic position as defined in claim 21.

24. A library of nucleic acids, each of which comprises one or more polymorphic positions within the human ACE gene, wherein said polymorphic positions are selected from the group consisting of positions in the ACE regulatory region numbered 5106, 5349, and 5496; positions in the ACE coding region numbered 375, 582, 731, 1060, 1215, 2193, 2328, 2741, 3132, 3387, 3503, and 3906; and position 1451 in the ACE gene as numbered in Genbank entry X62855.

25. A library as defined in claim 24, wherein the sequence at said polymorphic position in the regulatory region is selected from the group consisting of 5106C, 5106T, 5349A, 5349T, 5496T, and 5496C; and the sequence at said polymorphic position in the coding region is selected from the group consisting of 375A, 375C, 582C, 582T, 731A, 731G, 1060G, 1060A, 1215C, 1215T, 2193G, 2193A, 2328A, 2328G, 2741G, 2741T, 3132C, 3132T, 3387T, 3387C, 3503G, 3503C, 3906G, 3906A, and a deletion of nucleotides 1451-1783 as numbered in Genbank entry X62855.

26. A library of nucleic acids, each of which comprises one or more polymorphic positions within the human AGT gene, wherein said polymorphic position is selected from the group consisting of positions in the regulatory region numbered numbered 395, 412, 432, 449, 692, 839, 1007, 1072, 1204, and 1218; positions in the coding region numbered 273, 620, 803, 912, 997, 1116, and 1174; and position 49 in the AGT gene as numbered in Genbank entry M24688.

27. A library as defined in claim 26, wherein the sequence at said polymorphic position in the regulatory region is selected from the group consisting of 395T, 395A, 412C, 412T, 432G, 432A, 449T, 449C, 692C, 692T, 839G, 839A, 1007G, 1007A, 1072G, 1072A, 1204C, 1204A, 1218A, 1218G; and the sequence at said polymorphic position in the coding region is selected from the group consisting of 273C, 273T, 620C, 620T, 803T, 803C, 912C, 912T, 997G, 997C, 1116G, 1116A, 1174C, 1174A, and A or G at position 49 in Genbank entry M24688.

28. A library of nucleic acids, each of which comprises one or more polymorphic positions within the human AT1 gene, wherein said polymorphic position is selected from the group consisting of positions in the regulatory region numbered 1427, 1756, 1853, 2046, 2354, 2355, and 2415; and positions in the coding region numbered 449, 678, 1167, and 1271.

29. A library as defined in claim 28, wherein the sequence at said polymorphic position in the regulatory region is selected from the group consisting of 1427A, 1427T, 1756T, 1756A, 1853T, 1853G, 2046T, 2046C, 2354A, 2354C, 2355G, 2355C, 2415A and 2415G; and the sequence at said polymorphic position in the coding region is selected from the group consisting of 449G, 449C, 678T, 678C, 1167A, 1167G, 1271A, and 1271C.

30. A library of polymorphic patterns in the human ACE, AGT, and/or AT1 genes, comprising a member selected from the group consisting of: ACR 5349 A/T, AGR 1218 A; ACR 5496 C, AGR 1204 A/C; ACR 5496 C/T, AGR 1218 A, AGT 620 C/T; ACE 2193 A, AGR 1204 C, ACE 2328 G; ACE 2193 A, AGR 1204 A/C; ACE 3387 T, AGR 1218 A; ACE 3387 T, AGT 620 C/T; AGR 1204 A/C, AT1 678 C/T; AGR 1204 A/C, AT1 1271 A/C; ACE 1215 C, AGR 1204 A/C; AGR 1204 A/C, AT1 1167 A, ACE 3906 A/G; AGR 1204 A, AGT 620 C, AT1 1271 A, AT1 1167 A, AGR 395 A/T; AGR 1204 A/C, AGT 620 C/T, AT1 1271 A/C, AT1 1167 A, AGR 395 T; AGR 1204 A/C, AGT 620 C/T, AT1 1271 A/C, AT1 1167 A/G, AGR 395 T; AGR 1204 A, AT1 678 C, AT1 1167 A, AGR 395 A/T; AGR 1204 A/C, AT1 678 C/T, AT1 1167 A, AGR 395 T; AGT 620 C/T, AT1 1271 A/C, AT1 1167 A, AGR 395 T; AGT 620 C/T, AT1 1271 A/C, AT1 1167 A/G, AGR 395 T; AGT 620 C, AT1 1271 A, AT1 1167 A, AGR 395 A/T; AGT 620 C, AT1 678 A, AT1 1167 A, AGR 395 A/T; AGT 620 C/T, AT1 678 C/T; AT1 1167 A, AGR 395 T; ACE 2193 A, AGR 1218 A, AGT 803 A; ACE 2193 A, AGT 620 C/T; ACE 2328 G, AGT 620 C/T; ACE 3387 T, AGR 1204 A/C; ACE 2193 A, ACE 2328 G, AGR 1204 C; and ACE 2193 A/G, AGR 1072 G/G, AT1 1167 A/A.

31. A library of targets for cardiovascular drugs, each of said targets comprising an isolated peptide comprising one or more polymorphic positions in the ACE polypeptide sequence, wherein said polymorphic positions are encoded by nucleotides selected from the group consisting of nucleotide positions in the ACE coding region numbered 375, 582, 731, 1060, 1215, 2193, 2328, 2741, 3132, 3387, 3503, and 3906.

32. A library of targets for cardiovascular drugs, each of said targets comprising an isolated peptide comprising one or more polymorphic positions in the AGT polypeptide, wherein said polymorphic positions are encoded by nucleotides selected from the group consisting of nucleotide positions numbered 273, 620, 803, 912, 997, 1116, and 1174.

33. A library of targets for cardiovascular drugs, each of said targets comprising an isolated peptide comprising one or more polymorphic positions in the AT1 polypeptide, wherein said polymorphic positions are encoded by nucleotides selected from the group consisting of nucleotide positions numbered 449, 678, 1167, and 1271.

34. A kit for assessing cardiovascular status, said kit comprising

(i) sequence determination primers and

(ii) sequence determination reagents,

wherein said primers are selected from the group consisting of primers that hybridize to polymorphic positions in human ACE, AGT, or AT1 genes; and primers that hybridize immediately adjacent to polymorphic positions in human ACE, AGT, or AT1 genes.

35. A kit as defined in claim 34, wherein said polymorphic positions are selected from the group consisting of positions in the ACE regulatory region numbered 5106, 5349, and 5496; positions in the ACE coding region numbered 375, 582, 731, 1060, 1215, 2193, 2328, 2741, 3132, 3387, 3503, and 3906; position 1451 in the ACE gene as numbered in Genbank entry X62855; positions in the AGT regulatory region numbered 395, 412, 432, 449, 692, 839, 1007, 1072, 1204, and 1218; positions in the AGT coding region numbered 273, 620, 803, 912, 997, 1116, and 1174; position 49 in the AGT gene as numbered in Genbank entry M24688; positions in the AT1 regulatory region numbered 1427, 1756, 1853, 2046, 2354, 2355, and 2415; positions in the AT1 coding region numbered 449, 678, 1167, and 1271; and combinations of any of the foregoing.

36. A kit for assessing cardiovascular status, said kit comprising one or more antibodies specific for a polymorphic position within the human ACE, AGT, or AT1 polypeptides.

37. A kit as defined in claim 36, wherein said polymorphic positions are encoded by a nucleotide selected from the group consisting of nucleotide positions in the ACE coding region numbered 375, 582, 731, 1060, 1215, 2193, 2328, 2741, 3132, 3387, 3503, and 3906; nucleotide positions in the AGT coding region numbered 273, 620, 803, 912, 997, 1116, and 1174; positions in the AT1 coding region numbered 449, 678, 1167, and 1271; and combinations of any of the foregoing.